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SYNTHETIC AND CONFORMATIONAL STUDIES OF OLIGOSACCHARIDES RELATED TO GANGLIOSIDES

by

Subramaniam Sabesan

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Dedicated to

my family and teachers

ABSTRACT

The oligosaccharide portions of the gangliosides asialo-G_{M2} (βDGalNAc(1→4)βDGal(1→4)βDGlc-OCeramide) and asialo-G_{M1} (βDGal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlc-OCeramide) were synthesized as their 8-methoxycarbonyloctyl glycosides suitable for the preparation of artificial antigens and immunoadsorbents. The key steps in these syntheses involved the glycosylation of the 4'-hydroxyl group of suitably protected lactose derivatives with the appropriate acetylated 2-deoxy-2-phthalimido glycosyl bromides.

The conformational properties of the synthetic tri- and tetrasaccharides were examined by nuclear magnetic resonance (nmr) including the determination of ^{1}H- and ^{13}C-chemical shifts and ^{1}H-$\{^{1}H\}$ nuclear Overhauser enhancements.

The ^{13}C-chemical shifts of a biological sample of G_{M1} (βDGal(1→3)βDGalNAc(1→4)[αDNeuNAc(2→3)]βDGal(1→4)βDGlc-O-Ceramide) can be rationalized on the basis of those observed for the synthetic tetrasaccharide and methyl N-acetyl-αD-neuraminic acid. This observation indicates that no important conformational change occurs on sialylation of the 3'-position of asialo-G_{M1}.

Through the use of computer assisted hard-sphere exo-anomeric (HSEA) calculations, molecular models were produced for the oligosaccharide portions of *asialo*-G_{M1} and G_{M1} which are in accord with the observed nmr parameters. These models are presented as their CPK projections and are discussed in terms of the topographical features which are expected to mediate their interaction with antibody and lectin combining sites.

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I feel extremely fortunate to have worked under the guidance of Professor R. U. Lemieux. He created in me a sense of purpose and excitement in this project and provided numerous valuable ideas and advice. It is not easy to adequately express my gratitude to him. I am very thankful to Professor Klaus Bock for his collaboration on computer-assisted HSEA calculations and drawings. Dr. Ole Hindsgaul cheerfully gave his time to read this thesis and provide constructive criticism. As well, he was also very helpful in many other areas of this research. I also wish to thank Dr. A. Venot for proofreading this thesis and his suggestions as well as Dr. R. M. Ratcliffe and Professor S. Kundu for providing some of the compounds required in this project, Mr. Glen Bigam of the High Field Nmr Laboratory for his assistance, the staff in the Micro Analytical Laboratory, and all my fellow co-workers for their help.

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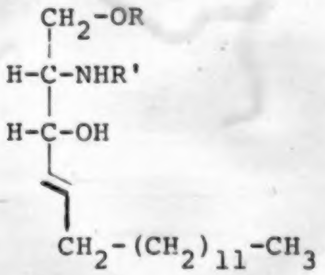
I. INTRODUCTION

A. Gangliosides

The substances known as gangliosides are a group of acidic glycolipids that were first characterized by Klenk[1]. He isolated these compounds from the brains of patients suffering from amaurotic familial idiocy and Nieman Pick disease.[2,3] Since Klenk could demonstrate the presence of these glycolipids only in the grey matter, he named these gangliosides.[4] It was not until 1953 that the presence of gangliosides in cells other than that of grey matter was established.[5] Subsequently in 1956, Svennerholm demonstrated the presence of more than one type of ganglioside in brain cells by a modified extraction procedure.[6]

The isolation, purification and structure elucidation of the gangliosides was first investigated by three independent groups led by Klenk,[1,7,8] Svennerholm,[6,9-11] and Kuhn and Wiegundt.[12,13] Based on acid hydrolysis and partial methylation studies, Svennerholm proposed in 1962 the sequence of the sugar units in the neutral cores of the two gangliosides known as GM_1 and GM_2. In 1963, Kuhn and Wiegandt[13] elegantly accomplished the complete structure elucidation of the substance which had been termed GM_1. Since then, the field has attracted many workers and to date the structures of over forty different gangliosides are known.[14]

It is now well established that the gangliosides (1) are complex glycosides of the lipid known as ceramide (1 with R = H, R' = $-\overset{O}{\overset{\|}{C}}-(CH_2)_n-CH_3$) which is a derivative of sphingosine where both R and R' of structure 1 are hydrogens.



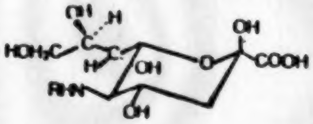
R = oligosaccharide

R' = $-\overset{O}{\overset{\|}{C}}-(CH_2)_n-CH_3$

1

The oligosaccharide portion of the ganglioside is composed of neutral sugar units that are glycosidically linked to form a linear core structure to which one or more residues of the acid ketose, known as *N*-acetyl neuraminic acid (NeuNAc residues), are glycosidically attached.

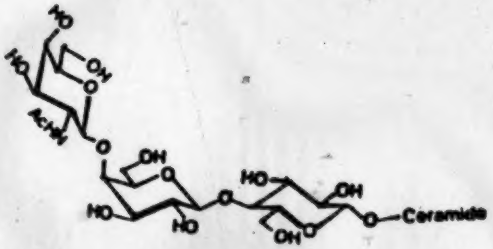
All the gangliosides isolated so far from the brain tissues contain only *N*-acetyl neuraminic acid (2) whereas the same gangliosides of membrane erythrocytes have been reported to contain *N*-glycolyl neuraminic acid (3).[15] Removal of the NeuNAc residues of gangliosides provides the neutral glycolipids known as *asialo* gangliosides. The term "asialo" was derived from the term sialic acid which is a



HOH₂C
OH
H
H
OH
OH
COOH
RHN
OH

2, $R = -\overset{O}{\overset{\|}{C}}-CH_3$: N-acetyl neuraminic acid (NeuNAc)

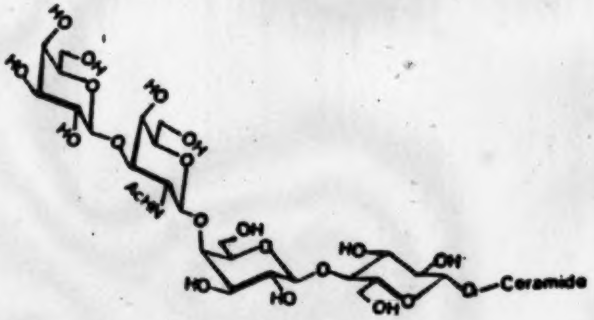
3, $R = \overset{O}{\overset{\|}{C}}-CH_2OH$: N-glycolyl neuraminic acid (NeuNGc)



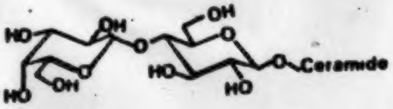
HO
HO
OH
AcHN
OH
HO
HO
HO
OH
OH
Ceramide

5, Asialo-G_{M2}

βDGalNAc(1→4) βDGal(1→4) βDGlc-OCer



HO
HO
OH
HO
HO
OH
AcHN
OH
HO
HO
HO
OH
OH
Ceramide



HO
OH
OH
HO
OH
HO
HO
Ceramide

4, Asialo-G_{M3}

βDGal(1→4) βDGlc-OCer

6, Asialo-G_{M1}

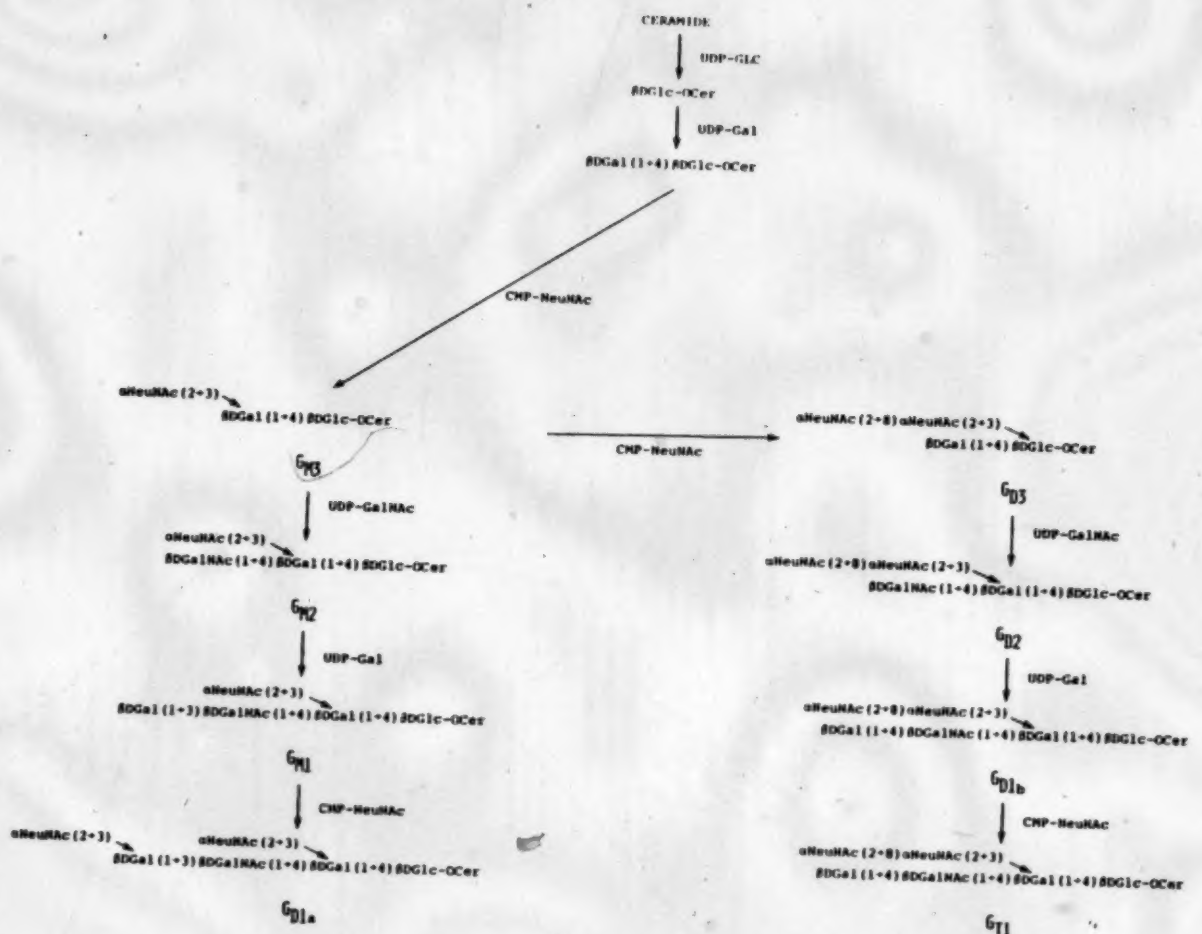
βDGal(1→3) βDGalNAc(1→4) βDGal(1→4) βDGlc-OCer

Fig. 1. Structures 2 (NeuNAc) and 3 (NeuNGc) are constituents of brain and erythrocyte gangliosides, respectively. Structures 4 to 6 form the oligosaccharide portions of asialo-gangliosides.

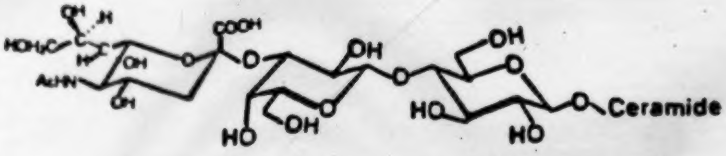
trivial name for both *N*-acetyl neuraminic acid and *N*-glycolyl neuraminic acid. The core oligosaccharides of *asialo* gangliosides begin with a β-lactosyl unit (βDGal(1→4)βDGlc) to which a βDGal unit or a βDGal(1→3)βDGalNAc unit is attached to the 4-position of the βDGal unit of the lactose residue (4 - 6). The sialic acid residues are attached to these core structures in two patterns; namely, the 3-position of the Gal unit of the lactose or the terminal galactose of the structure 6 or both.

This thesis is concerned only with the gangliosides which contain the NeuNAc form of sialosides. These gangliosides have been found to contain 1 to 7 NeuNAc residues. In the case of brain tissues, an average of 2 - 2.5 NeuNAc residues are present.[14] The composition of gangliosides is exemplified by the structures which are presented in Scheme I in terms of their biosynthetic pathway.[16] The structures termed GD_3, GD_2 and GD_{1b} illustrate the fact that the gangliosides can contain oligomers of NeuNAc as side chains of the neutral core.

As shown in Scheme I, the NeuNAc residues are present as α-D-glycosides. This structural feature can be appreciated from the structural formula 7 for the simple ganglioside GM_3.


CERAMIDE
UDP-GLC
βDGlc-OCer
UDP-Gal
βDGal(1→4)βDGlc-OCer
CMP-NeuNAc
αNeuNAc(2→3)
βDGal(1→4)βDGlc-OCer
GM3
CMP-NeuNAc
αNeuNAc(2→8)αNeuNAc(2→3)
βDGal(1→4)βDGlc-OCer
GD3
UDP-GalNAc
αNeuNAc(2→3)
βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GM2
UDP-GalNAc
αNeuNAc(2→8)αNeuNAc(2→3)
βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GD2
UDP-Gal
αNeuNAc(2→3)
βDGal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GM1
UDP-Gal
αNeuNAc(2→8)αNeuNAc(2→3)
βDGal(1→4)βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GD1b
CMP-NeuNAc
αNeuNAc(2→3)
αNeuNAc(2→3)
βDGal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GD1a
CMP-NeuNAc
αNeuNAc(2→8)αNeuNAc(2→3)
βDGal(1→4)βDGalNAc(1→4)βDGal(1→4)βDGlc-OCer
GT1

Scheme 1. Biosynthesis of gangliosides reproduced from an article, "Biosynthesis of Gangliosides", by Brady and Fishman.[16]



GM$_3$ (αDNeuNAc(2→3)βDGal(1→4)βDGlc-OCer

7

It is noteworthy that the α-sialosides have the carboxyl group in axial orientation. As a consequence, the glycosidic linkage is extremely acid labile. Also, this structural feature contributes the problem of establishing the α-glycosidic linkage by chemical means.

The nomenclature used in the biosynthetic Scheme I will be used throughout this thesis. This nomenclature was first presented in 1963 by Svennerholm.[11,17] This terminology uses "G" to state that the structure is a ganglioside and the terms M (mono), D (di), T (tri), etc., are to indicate the number of NeuNAC residues. The arabic numbers 1 to 3 indicate the number of sugars in the neutral core chain, except that for historical reasons these numbers are used in reverse in that 1 refers to the tetrasaccharide (6), 2 to the trisaccharide (5) and 3 to the lactosyl structure (4).

The lower case letters a and b are used only to name gangliosides when these are derived from the tetrasaccharide neutral core. Since the NeuNAc residues are found to occur only at the 3-positions of βDGal units, the letter 'a' is used to indicate the presence of only one NeuNAc residue at the 3'-position of the βDGal unit of the lactosyl group. When the ganglioside has a dimer of NeuNAc at this position, the letter 'b' is used, and so on. Besides the above nomenclature system, the reader should be aware of the recent recommendation by the IUPAC-IBC commission for the designation of gangliosides.[18] For example, according to this system, the ganglioside G_{M1} would be referred to as II^3 NeuNAcGgOSe$_4$.

B. Occurrence and Distribution of Gangliosides

Although most of the gangliosides isolated so far are from the brain tissues, these compounds are widely distributed in the tissues of mammals and perhaps all vertebrates.[14] In adults, the grey matter contains as much as three times more gangliosides than those which are contained by the white matter. As illustrated in Table 1, the gangliosides G_{M1} and higher members are present to a greater extent than the gangliosides G_{M2} or G_{M3}.[17]

The relationship of the gangliosides to other glycolipids that occur in tissues is, in part, presented in

Table 1. The ganglioside content in brain tissues of vertebrates, reproduced from an article, "Gangliosides", by L. Svennerholm.[17]

Material	Total NAN µg/g wet weight	Distribution of NAN%							
		G_{M3}	G_{M2}	G_{D3}	G_{M1}	G_{D1a}	G_{D1b}	G_{T1}	G_{Q1}
Human									
Cerebral cortex									
Fetus 5 months	300								
Newborn	400	1.0	3.6	1.1	14.6	71.6	1.8	7.3	
2.5 months–8 yr (6 cases)	738	1.0	2.3	4.0	14.2	42.5	12.7	15.9	3.6
3–5 yr (7 cases)	686								
8 yr	797		3.6		17.4	39.4	19.8	15.9	2.9
44 yr	1.002		1.3		11.3	22.4	28.3	29.9	5.9
73 yr	796		1.7		12.8	22.8	25.5	31.2	5.1
60–90 yr (40 cases)	846								
White matter									
Newborn	400	1.0	6.9	1.4	19.1	57.8	2.1	3.4	
2.5 months–8 yr (6 cases)	60	1.0	1.5	1.5	16.1	38.5	11.6	18.1	8.2
3–5 yr (7 cases)	269								
8 yr	73		1.7		20.4	42.9	12.5	16.0	5.9
44 yr	156		1.0		9.3	14.0	31.4	38.2	6.4
73 yr	191		1.9		12.6	18.4	30.4	27.9	7.9
60–90 yr (16 cases)	312								
Ox									
Cerebral cortex	940								
	497		2.0		15	39	15	22	6
White matter	210								
	102				26	35	15	20	4
Rat (adult)	1.047		1.2		13.0	32.1	20.3	27.3	6.0
Mice	640								
Guinea pig	425								
Rabbit									
Cerebral cortex	497		3		15	38	16	23	5
Hen	590								
Chicken	590								
Chicken embryos									
5 days	23								
8 days	119								
13 days	233								
18 days	314								
21 days	605								
Chicken hatched 2 days	660								
Turtle	220								
Snake	320								
Frog	220								
Fish	370								

Scheme 2. It is seen that, in addition to gangliosides, lactosyl ceramide (Lac-Cer) serves as a building unit of many different kinds of glycosphingolipids.

C. Function of Gangliosides

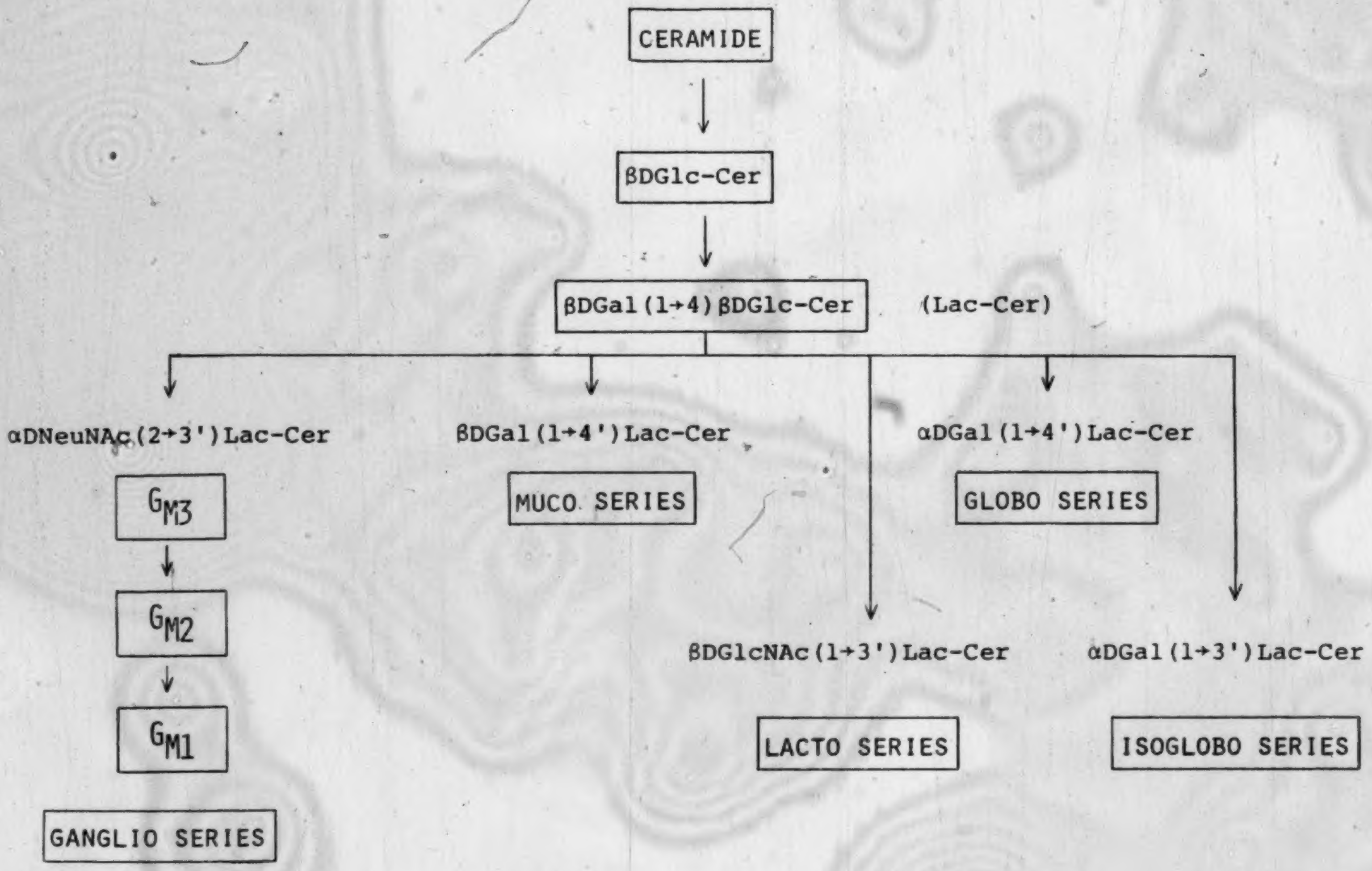
1. Receptor for neurotransmitters

The presence of at least one sialic acid residue appears to be one of the requirements for many of the observed biological functions of gangliosides.[19] Since the gangliosides are localized at the nerve terminals, it has been proposed[20] that these provide receptor sites for the cations, especially the calcium ions. Also, it has been found that there is a strong and highly specific interaction between gangliosides and serotonin.[21] Besides serotonin, gangliosides have been reported to bind tryptamine, lysergic acid diethyl amide, ergotometrine, strychnine and brucine, all of which are known to affect the central nervous system.[21]

2. Gangliosides as cell-surface membrane receptors

The interaction between gangliosides and a protein or glycoprotein is one of the most extensively studied areas of ganglioside chemistry.[22]

Van Heyningen[23,24] first reported that the ganglioside G_{M1} binds cholera toxin and thereby blocks its physiological action. Later, by chemical modification and labelling


CERAMIDE
βDGlc-Cer
βDGal(1→4)βDGlc-Cer
(Lac-Cer)
αDNeuNAc(2→3')Lac-Cer
G_{M3}
G_{M2}
G_{M1}
GANGLIO SERIES
βDGal(1→4')Lac-Cer
MUCO SERIES
βDGlcNAc(1→3')Lac-Cer
LACTO SERIES
αDGal(1→4')Lac-Cer
GLOBO SERIES
αDGal(1→3')Lac-Cer
ISOGLOBO SERIES

Scheme 2: Classification of Glycosphingolipids studies, other independent groups[25-27] confirmed this conclusion. The toxin seems to be very specific for G_{M1} even in the presence of other gangliosides.

Tetanus toxin (inhibitor of neurotransmitters at the nerve terminals) was found to bind to the presynaptic nerve ending[28] and this has been attributed to the presence of the gangliosides G_{D1b}, G_{T1b} and G_{Q1b} which are rich in these membranes.[19,21] The maximum binding of the toxin by the gangliosides requires the presence of at least two sialic acid residues to be attached as oligomers to the inner galactose residue of the tetrasaccharide unit of asialo G_{M1}.

The binding capacity of gangliosides with toxins seems to exhibit a remarkable structural specificity. For example, in contrast to tetanus toxin, the binding of the sendai virus needs two sialic acid residues in the outer galactose of the neutral oligosaccharide core.[29,30] This virus agglutinates human erythrocytes. Since the agglutination is inhibited by gangliosides,[29] the presence of the gangliosides is indicated. Also, there are reports which suggest that the receptor site for human and fibroblast interferon[22] and botulinum toxin[22] are bound by carbohydrate portions of gangliosides.

Gangliosides are established to bind glycoprotein hormones[32,33] including the thyroid stimulating hormone, luteinizing hormone, human chorionic gonadotropin and follicle stimulating hormone. This subject has recently been reviewed by Brady et al.[16] and by Kohn et al.[22]

A detailed study of the mechanism of ganglioside interaction with hormones and toxins indicates a remarkable similarity in the amino acid sequence in the receptor site of these glycoprotein molecules. The available evidence[16] suggests that complex formation activates the enzyme adenylate cyclase. Brady and Fishman[16] have suggested a possible correlation between the adenylate cyclase activity and the cell growth. A change in composition of gangliosides which has been observed in many human cells may make the adenylate cyclase enzyme in these cells less sensitive to undetermined physiologic stimuli that interact with ganglioside receptors. These stimuli may be serum factors in substances secreted by cells that are involved in the regulation of the growth of normal cells. In addition, membrane components on the surface of the cell could interact specifically with surface gangliosides of another cell. This association would lead in turn to a stimulation of adenylate cyclase and the subsequent change in cell growth.

Finally, it is of interest to note that the immunity of the natural killer cell against the viral attack has been related to the presence of asialo G_{M1}.[34] It has been reported that the treatment of these killer cells with anti-asialo-G_{M1} antibody results in a reduction of the killer cell activity and an enhancement of the tumor growth.

D. Disease

1. Cancer

A number of reviews have appeared in recent years[35-38] which show that oncogenic transformations may be accompanied with changes in the glycolipids present on the plasma membranes. In this regard, asialo-G_{M2} has been implicated as a tumor associated antigen since it could be detected at the surface of certain tumor cells.[39] Preliminary experiments indicate[40] that the treatment of these tumor cells with anti-asialo-G_{M2} antibodies completely suppressed the tumor growth.

The simplest member of the ganglioside family, asialo-G_{M3} (βDGal(1$\rightarrow$4)βDGlc-Cer) has been implicated as a cell surface marker of human neutrophils[41] and found at increased levels in human mylogenous leukemia cells.[42] The disialosyl gangliosides G_{D2} and G_{D3} have been characterized as tumor markers in cells of melanoma and other human tumors.[43,44] Interestingly, an unusual fucoganglioside (fuco-asialo-G_{M1}) with blood group activity has been detected in highly malignant rat hepatoma cells.[45]

2. Gangliosidosis

A number of neurological disorders are accompanied by a disturbance in the ganglioside pattern in brain tissues.[46] One of the earliest discoveries is the "infantile

G_{M2} gangliosidosis" which results in the accumulation of G_{M2} and asialo-G_{M2} in nervous tissues.[46] Similarly, accumulation of G_{M1} (G_{M1} gangliosidosis) results in a neurological disorder in infants.[46] These storage diseases are due to the absence of certain glycosidase enzymes which are involved in the catabolism of gangliosides.[47,48]

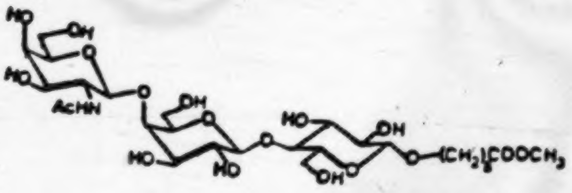
3. Cholera

It has been proven beyond any doubt that this disease results from the specific binding of the cholera toxin to the ganglioside G_{M1}.[49]

E. Synthetic Goals

In order to better understand the biological significance of these gangliosides, an acceptable supply of these materials is needed. The availability of these materials from biological sources is very limited. Furthermore, the substances are often contaminated by other members present in the same biological source and which cannot be removed by presently available techniques for separation. This is especially a serious problem in the immunochemical studies of gangliosides with specific antibodies.[50] Chemical synthesis can, in many cases, be expected to provide well-defined chemical structures in adequate amounts.[51]

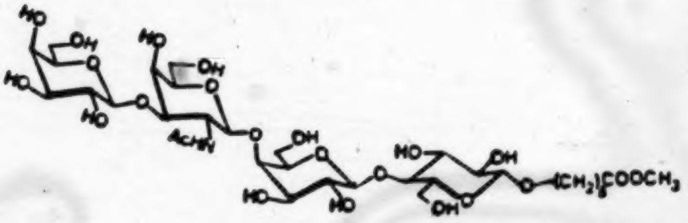
With the above possibilities in mind, it was planned to synthesize the oligosaccharides related to the asialo-G_{M2} and asialo-G_{M1} gangliosides in the forms presented in structures 8 and 9. The purpose of the aglycon $-(CH_2)_8-COOCH_3$ is to serve as a linking arm, as was demonstrated by Lemieux and coworkers for the preparation of artificial antigens[52] and immunoadsorbents.[53] Structures 8 and 9 may be used for the preparation of immunochemical reagents related to the G_{M1} and G_{M2} gangliosides as was shown by


HO
OH
HO
AcHN
OH
HO
HO
HO
OH
OH
$(CH_2)_8COOCH_3$

Asialo-G_{M2} Hapten

βDGalNAc(1→4)βDGal(1→4)βDGlc-O$(CH_2)_8COOCH_3$

8


HO
OH
HO
HO
HO
OH
AcHN
OH
HO
HO
HO
OH
OH
$(CH_2)_8COOCH_3$

Asialo-G_{M1} Hapten

βDGal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlc-O$(CH_2)_8COOCH_3$

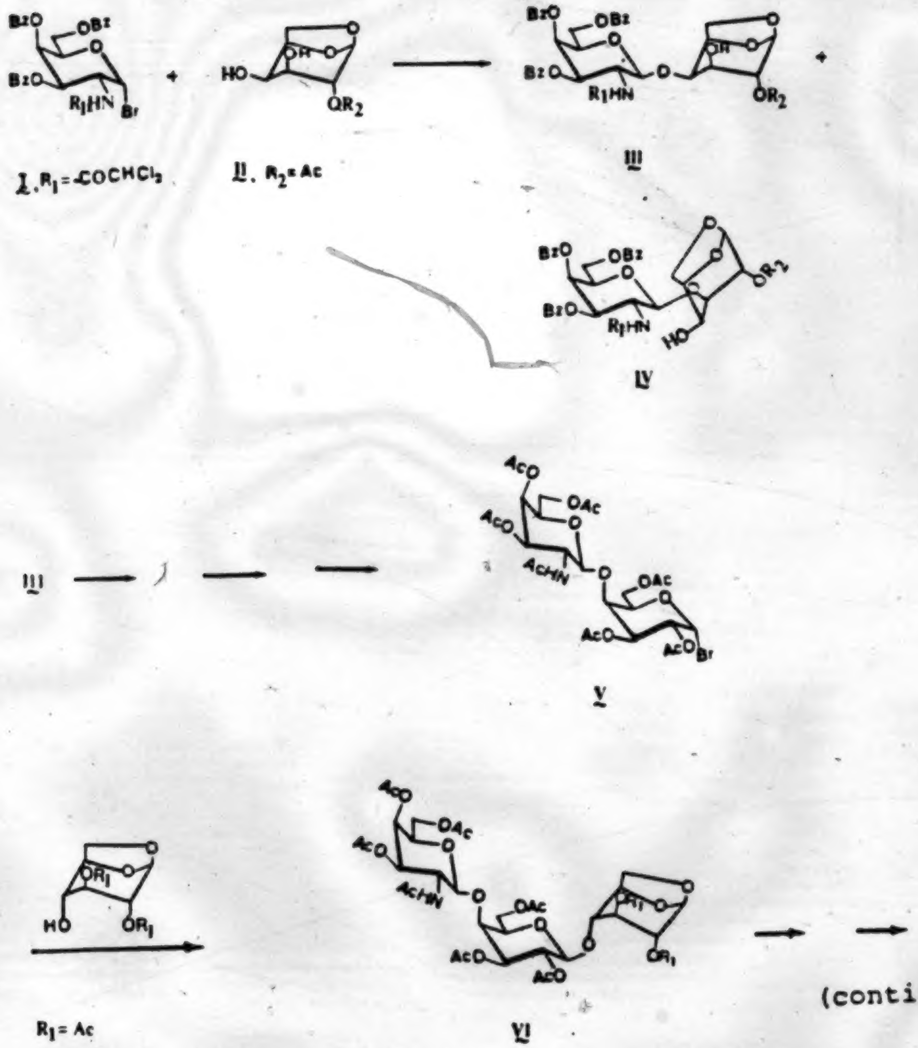
9

Lemieux et al.[54] for the preparation of antibodies specific for Lewis blood group determinants.

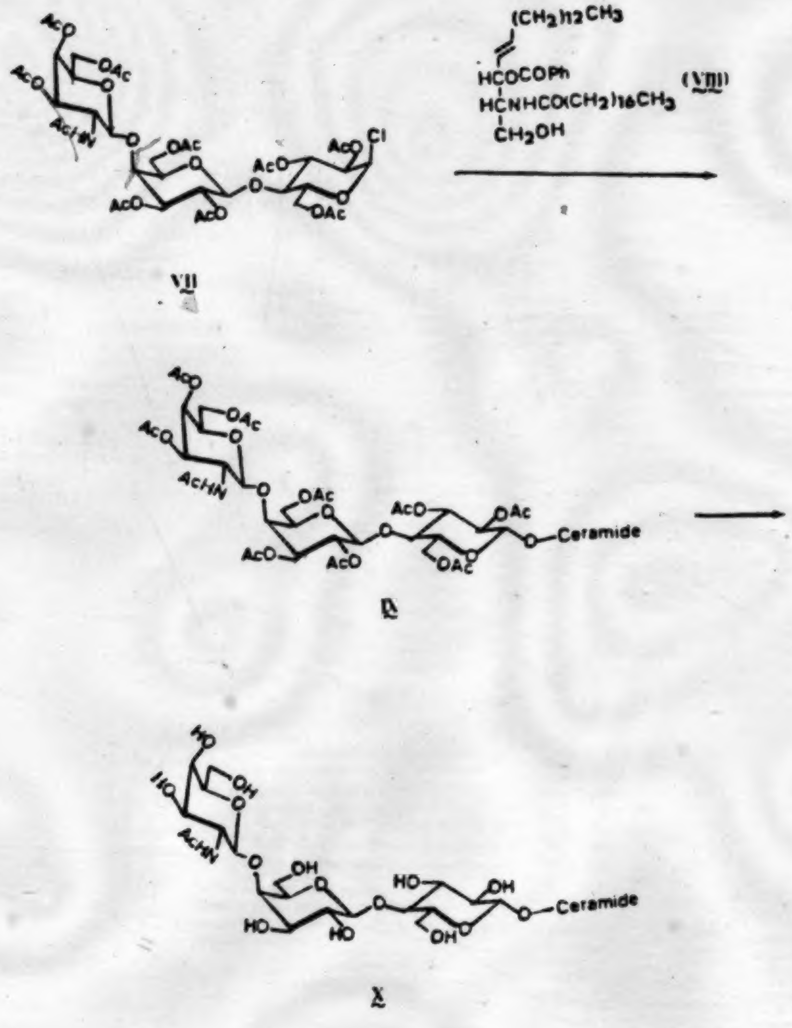
F. The Synthetic Methods

This section is provided in the form of an outline of the synthetic program that was followed, since this procedure will serve as a convenient means to indicate the origins of the synthetic methodologies that were used in the overall program. Thus, the literature survey will also serve to acquaint the reader with the subject of the thesis.

It is to be noted that Shapiro and Acher[55,56] reported the total synthesis of asialo-G_{M2} in 1973. The procedure used in this pioneering work is outlined in Scheme 3. The use of the dichloroacetyl protecting group in I was based on earlier observations[57] which demonstrated that the presence of powerful electron-withdrawing groups in the acetamido function rendered the bromide I more stable and less prone to reaction with neighbouring acylamino group participation in glycosylation reactions.[58] The diol II was employed because it was readily prepared and it was anticipated that the Koenigs-Knorr type reaction using mercuric cyanide as promoter (known as Helferich condition) would provide selective glycosylation of the equatorial hydroxyl group. In


I, R1 = COCHCl2
II, R2 = Ac
III
IV
V
R1 = Ac
VI

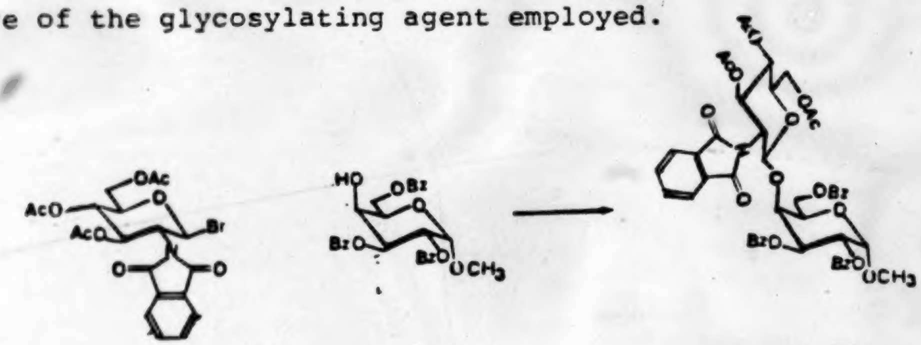
(continued)


(CH2)12CH3
HCOCOPh
HCNHCO(CH2)16CH3
CH2OH
(VIII)
AcO
OAc
AcHN
Cl
VII
Ceramide
IX
HO
OH
X

Scheme 3

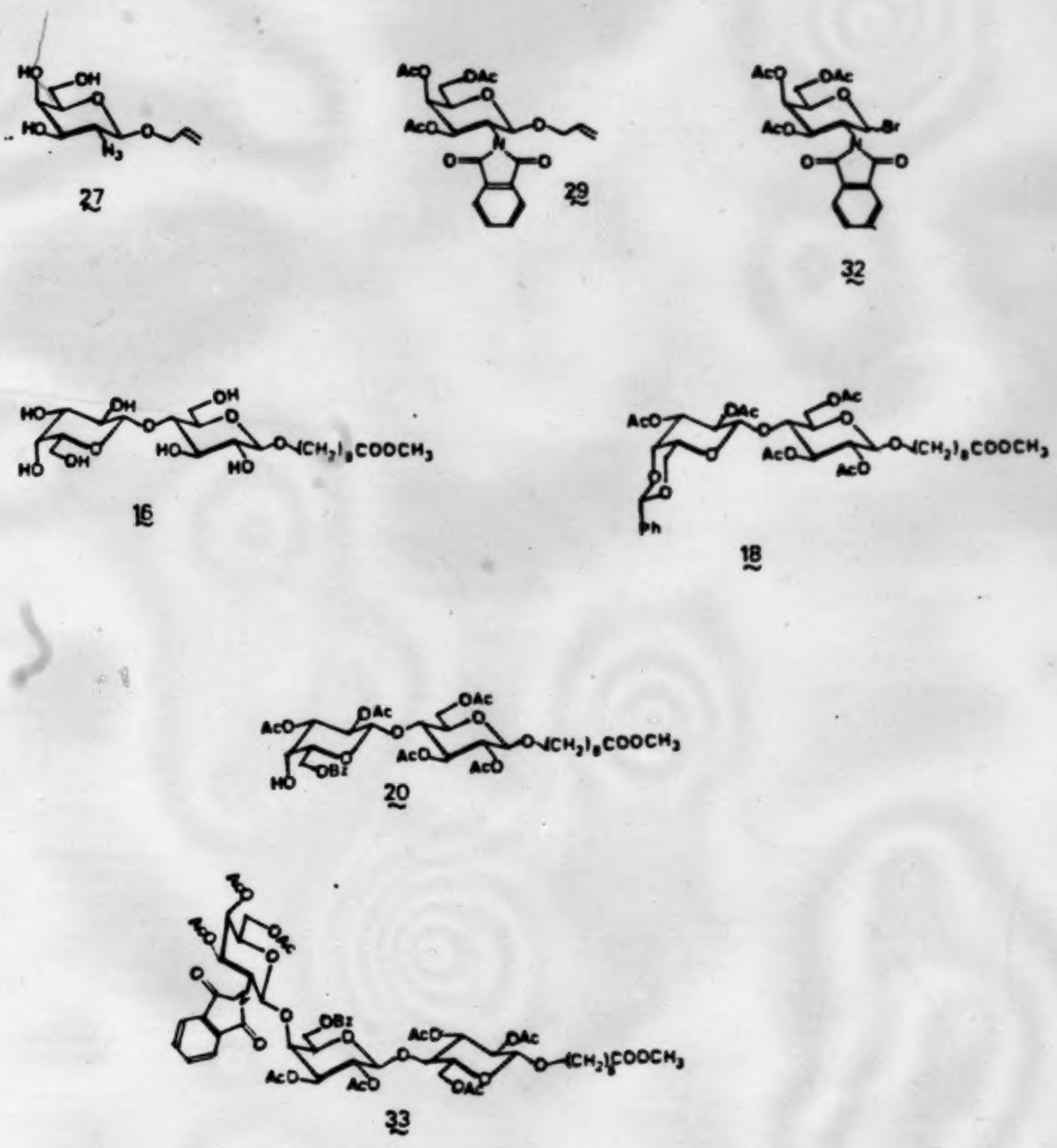
fact, the mixture of β-linked disaccharides III and IV was obtained in 45% yield in a 3:2 ratio.[59] These could be separated by column chromatography on silica gel but no yields were reported. Although the subsequent steps involved intermediates that were not well characterized, the structure of the target molecule asialo-G_{M2} (X) could be established by a comparison of the optical rotation and the relative mobility on silica gel of the synthetic material with that of the natural glycolipid.

The main challenge in terms of the synthesis of asialo-G_{M2} could have been expected to be the establishment of (1→4)β-linkage between the N-acetylgalactosamine and the galactose residue to form the terminal disaccharide portion. However, although a number of comments can be found in the literature[60] regarding the resistance to glycosylation of the 4-hydroxyl group of suitably protected galactose derivatives, there does not exist any definitive information in this regard. Shapiro and Acher[56] expressed an interest in the diol II because for this compound the 4-hydroxyl group is in equatorial orientation. However, as noted above, the axial 3-hydroxyl group proved to react only 1.5 times more slowly. On the other hand, it was demonstrated by Lemieux, Takeda and Chung[61] that the 4-hydroxyl of the galactose derivative X could be glycosylated with 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (XI) to provide the (1→4) linked β-disaccharide XII in about 80% yield. Therefore, the reactivity of the 4-hydroxyl group of the galactose derivatives appear to depend rather on the nature of the glycosylating agent employed.



XI X XII

The use of the phthalimido bromides which has been termed the 'phthalimido method' has since found wide application and appears to be a very reliable method of choice for the synthesis of a variety of 2-amino-2-deoxy-glycosides. Recently, a comparison in terms of yield and stereoselectivity was made[62] with conventional sugar oxazolines[63-65] and the advantages of the phthalimido method were displayed. Since it was anticipated that the reactivity of the 4-hydroxyl group of suitably blocked lactose derivatives would be of the same order as the alcohol X, it was decided to prepare 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide 32 which was required for the glycosylation of the alcohol 20 to provide the blocked asialo-G_{M2} hapten (33). This synthetic strategy can be appreciated by a consideration of the synthesis of the blocked trisaccharide 33.


27
29
32
16
18
20
33

Scheme 4

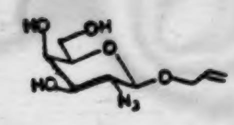
The required reagent 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide (**32**) could have been prepared from commercially available D-galactosamine following the procedures reported for the preparation of the corresponding *gluco* analogue.[66] However, since D-galactosamine is very expensive, allyl 2-azido-2-deoxy-β-D-galactopyranoside (**27**) was chosen as the starting material and this compound was expected to be available by the way of the azidonitration of D-galactal triacetate following the procedures reported by Lemieux and Ratcliffe.[67] Also, as will be seen later on, the allyl glycoside (**27**) was an attractive intermediate for the synthesis of *asialo*-G_{M1} hapten (**9**).

The choice of the allyl aglycon for **27** was to serve as a temporary protecting group, which could be readily removed under the mild conditions that were reported by Corey and Sugg,[68] and used by Gigg and Gent.[69] The reduction of the azide to amine and the protection of the functional groups, as seen in the intermediate **29**, was expected to be carried out under the conditions reported.[70,71] It was anticipated that the removal of the *O*-allyl protecting group and the treatment of the 1-hydroxyl compound with *N,N*-dimethylbromoforminium bromide (Vilsmeier bromide)[72] would provide the desired phthalimido bromide **32**.[70]

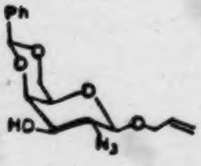
As outlined in Scheme 4, it was necessary to prepare the alcohol **20** for condensation with the bromide **32** to form the intermediate **33**. In this regard, the starting material was 8-methoxycarbonyloctyl β-lactoside (**16**), a compound which had already been reported by Banoub and Bundle[73] by condensation of β-lactose octaacetate with 8-methoxycarbonyloctanol in the presence of stannic chloride. The preparation of its 4',6'-O-benzylidine acetal (**17**) could be anticipated from the well documented procedures.[74,75] Acetylation would then yield the intermediate **18**. Preferential removal of the 4',6'-*O*-benzylidene group under the conditions reported by Metzner et al.[75] would provide the diol, the primary hydroxyl of which could be selectively benzoylated[75,76] to form the desired intermediate **20**.

Finally, the condensation of the phthalimido bromide (**32**) with the alcohol **20** using silver triflate-collidine complex[61] was expected to provide the blocked *asialo*-G_{M2} derivative **33**.

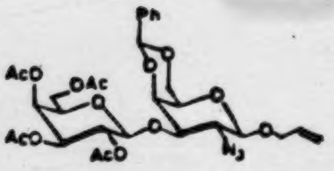
Similarly, the strategy in the synthesis of *asialo*-G_{M1} hapten (**9**) can be illustrated by considering the preparation of the blocked *asialo*-G_{M1} derivative **46**. It was expected that the alcohol **27** could be converted to the 4,6-*O*-benzylidene derivative **35** following well established procedures.[77] In fact, Lemieux and coworkers prepared[78] the


HO
OH
O
HO
O
N₃

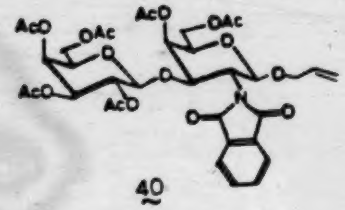
27


Ph
O
O
O
HO
O
N₃

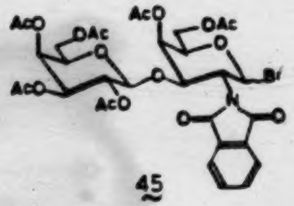
35


Ph
AcO
OAc
O
AcO
AcO
O
O
O
O
O
N₃

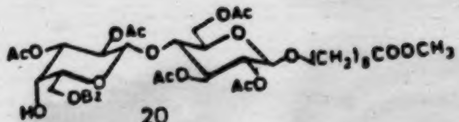
36


AcO
OAc
AcO
OAc
O
O
AcO
AcO
O
O
N
O
O

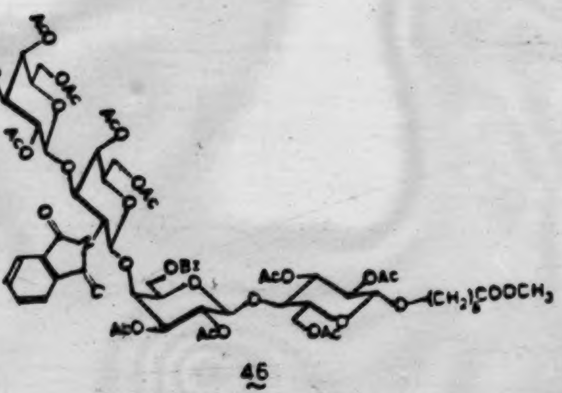
40


AcO
OAc
AcO
OAc
O
O
AcO
AcO
O
Br
N
O
O

45

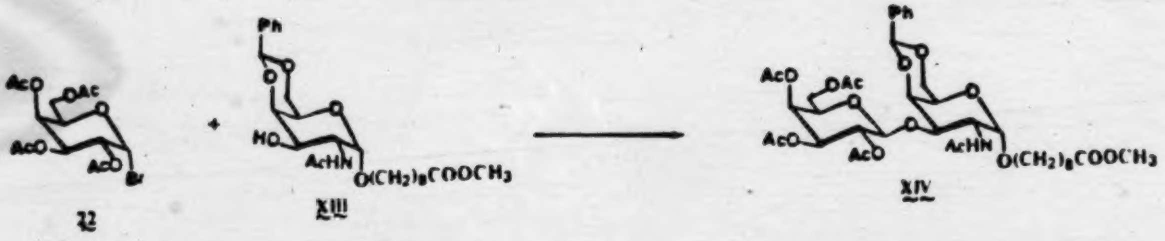

OAc
AcO
OAc
O
O
O
OBz
AcO
AcO
O(CH₂)₈COOCH₃
HO

20


AcO
AcO
OAc
O
AcO
O
AcO
OAc
O
O
N
O
OBz
O
AcO
OAc
O
(CH₂)₈COOCH₃
AcO
AcO
OAc

46

4,6-O-benzylidene derivative (XIII) of 8-methoxycarbonyloctyl-2-acetamido-2-deoxy-β-D-galactopyranoside in 73% yield using a standard procedure.[77] It could be expected that the glycosylation of 35 with acetobromogalactose under Helferich conditions would be successful since a similar


AcO
OAc
AcO
AcO
Br
22
+
Ph
O
O
O
HO
AcHN
O(CH2)8COOCH3
XIII
AcO
OAc
AcO
AcO
O
Ph
O
O
O
AcHN
O(CH2)8COOCH3
XIV

compound (XIV), a product related to the T-antigen βDGal(1→3)βDGalNAc, was prepared by Lemieux and coworkers under the above-mentioned conditions.[78]

Since the phthalimidohalide procedure for the preparation of β-glycosides of 2-amino-2-deoxy sugars, as was mentioned earlier, appeared attractive, the plan was to convert the allyl glycoside (36) to the phthalimido bromide (45). The procedure outlined for the conversion of 27 to 32 would be followed. This would involve de-O-benzylidenation of 36 followed by deacetylation, reduction of azide to amine, reaction of the amine with phthalic anhydride and acetylation to form the phthalimido glycoside 40. Removal of the allyl group[69] and treatment of the alcohol with Vilsmeier reagent[72] would then provide the desired reagent 45.

G. Conformational Analysis

As mentioned earlier, gangliosides which are present on plasma membranes have been implicated both in intracellular recognition processes and in the binding of regulatory molecules.[30] It has been proposed that these processes are mediated through complex formation by binding of a portion of the oligosaccharide of these glycolipids with the receptor site, normally referred to as the combining site, of the biologically active agent such as an antibody, toxin or glycoprotein hormone.[79] It can be expected that this binding is best achieved when both the combining site and the oligosaccharide portions are conformationally well-defined complementary structures. Since it can be anticipated that the carbohydrate ligands will be bound in a conformation that is near that which is energetically most favorable in aqueous solutions and since these cell surface oligosaccharides perform their functions in aqueous environments, an appreciation of their most likely solution conformations is a prerequisite for a meaningful interpretation of their biological functions. Consequently, experimental evidence is required to provide information regarding the conformational preferences of these oligosaccharides and, as has been pointed out by Lemieux,[80] modern high frequency nuclear magnetic resonance (nmr) spectroscopy can prove well suited for this purpose.

With the development of new synthetic methodologies,[81,82] it is now possible to obtain, through chemical synthesis, complex oligosaccharides in amounts adequate for nuclear magnetic resonance studies. In addition, the availability of highly sophisticated computer-assisted high frequency spectrometers, because of their great resolving power and the high stability of the applied magnetic field, has enabled the measurement of a number of nmr parameters which relate to the conformational properties of the molecule. These include spin-lattice relaxation times (T_1's) and nuclear Overhauser enhancements (nOe's) as well as chemical shifts and spin-spin coupling constants.

It is well recognized[83] that an nOe is dependent not only on the proximity of the proton observed to the proton which is saturated, but also on the immediate proton environment of the former. In rigid systems of known geometry and in which the molecules are tumbling isotropically so that the correlation time (τ_c) is the same for every proton vector and with intramolecular dipole-dipole relaxation predominating, the observed nOe for proton d on the saturation of s is well approximated for the purpose of estimating relative nOe's by the following expression:

$$nOe_d(s) = r_{ds}^{-6} / (2 \sum_{j \neq d} r_{dj}^{-6})$$

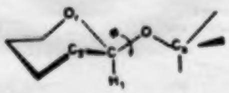
where r_{ds} is the distance between proton d and proton s and r_{dj} is the distance between proton d and each other proton in the molecule.

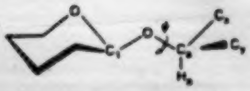
Under similar conditions, the spin-lattice relaxation time (T_1) is also dependent on the environment of a proton and can be expressed as follows:

$$(\frac{1}{T_1}) = \text{constant} \cdot \tau_c \cdot \sum_{j \neq i} r_{rj}^{-6} \quad .$$

In order to gain an appreciation of nOe and T_1 values, it was necessary to generate the molecular structures in the memory of a computer so that relevant internuclear distances could be conveniently extracted and used in calculations. The hard-sphere exo-anomeric effect (HSEA) calculations (see below) appear to be very useful in this regard since, to date, it has produced results in excellent agreement with the conformation expected from the nmr studies of oligosaccharides dissolved in heavy water.[84] This is perhaps remarkable since the procedure is arbitrary and artificial in many regards but, on the other hand, it encompasses the least number of assumptions and is based on a large body of experimental data.[80]

Even for oligosaccharides containing several sugar units, a sufficient number of coupling constants for vicinal protons can normally be measured so as to leave no doubt as to the conformation of the pyranose rings. Thus, the problem of determining conformational preferences for an oligosaccharide resides in establishing the values of torsion angles defined by vicinal atoms about the various glycosidic bonds. The torsion angles are defined as ϕ and ψ as presented below.[85]





$\phi^H = C_a/H_1 = \phi$

$\phi^O = C_a/O_r$

$\phi^C = C_a/C_2$

$\psi^H = C_1/H_a = \psi$

$\psi^{C_x} = C_1/C_x$

$\psi^{C_y} = C_1/C_y$

The procedure used in the HSEA calculations for estimating conformational preferences of oligosaccharides related to gangliosides will be that described by Lemieux, Bock and co-workers[80] for the B trisaccharide (βDGal(1→3)[αLFuc(1→2)]βDGal-OR). These HSEA calculations are based on the assumption that the sugar units can be treated as rigid bodies with the atomic co-ordinates from the appropriate crystal structures. The conformational preference for a glycosidic linkage is then gauged by taking into account nonbonded interactions between atoms taken as hard spheres of appropriate van der Waal's radii but including a contribution to the relative stabilities of conformers which differ only in their ϕ torsion angles and which arise because of the exo-anomeric effect.[86] It should be realized that a change in energy associated with a change in ϕ torsion angle is due to stereoelectronic considerations while that associated with ψ torsion angles is steric in origin. The hard-sphere interaction energy (HS) was calculated using the Kitaigorodsky expression:[87]

$$\mathrm{HS} = 30{,}000\, e^{-13d/d_o} - 0.14\,(d/d_o)^{-6}\ \text{kcal/mole}$$

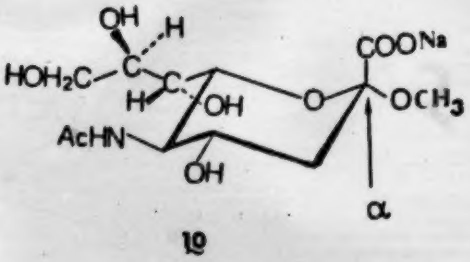
where d is the internuclear distance of two atoms and d_o is 1.1 times the sum of their van der Waal's radii.

The exo-anomeric effect (EA) for α and β anomers was then calculated by the expression synthesized by Thøgersen:[80]

$$EA^{\beta} = 1.66\ EA^{\alpha}\ \text{kcal/mole}$$

where $EA^{\alpha} = 1.58(1-\cos\phi°)-0.74(1-\cos 2\phi°)-0.70(1-\cos 3\phi°) + 1.72$ kcal/mole. It is evident from these expressions that the exo-anomeric effect contribution is expected to be greater for the β anomer.

It was our hope that the average minimum energy conformation provided by the HSEA calculations would explain the observed nOe, T_1, ^{13}C- and ^{1}H-nmr parameters of the synthetic asialo-G_{M2} (8) and asialo-G_{M1} (9) haptens. It was expected that a comparison of the ^{13}C-chemical shifts of 8, 9 and the sodium salt of α-methyl sialoside (10) with those of the reported values for the natural gangliosides[115,116] would provide a knowledge regarding the most probable solution conformation of these molecules. Such a knowledge might be extremely useful toward an understanding of the different binding specificities exhibited by various ganglioside molecules with antibodies, toxins and hormones.[30]



10

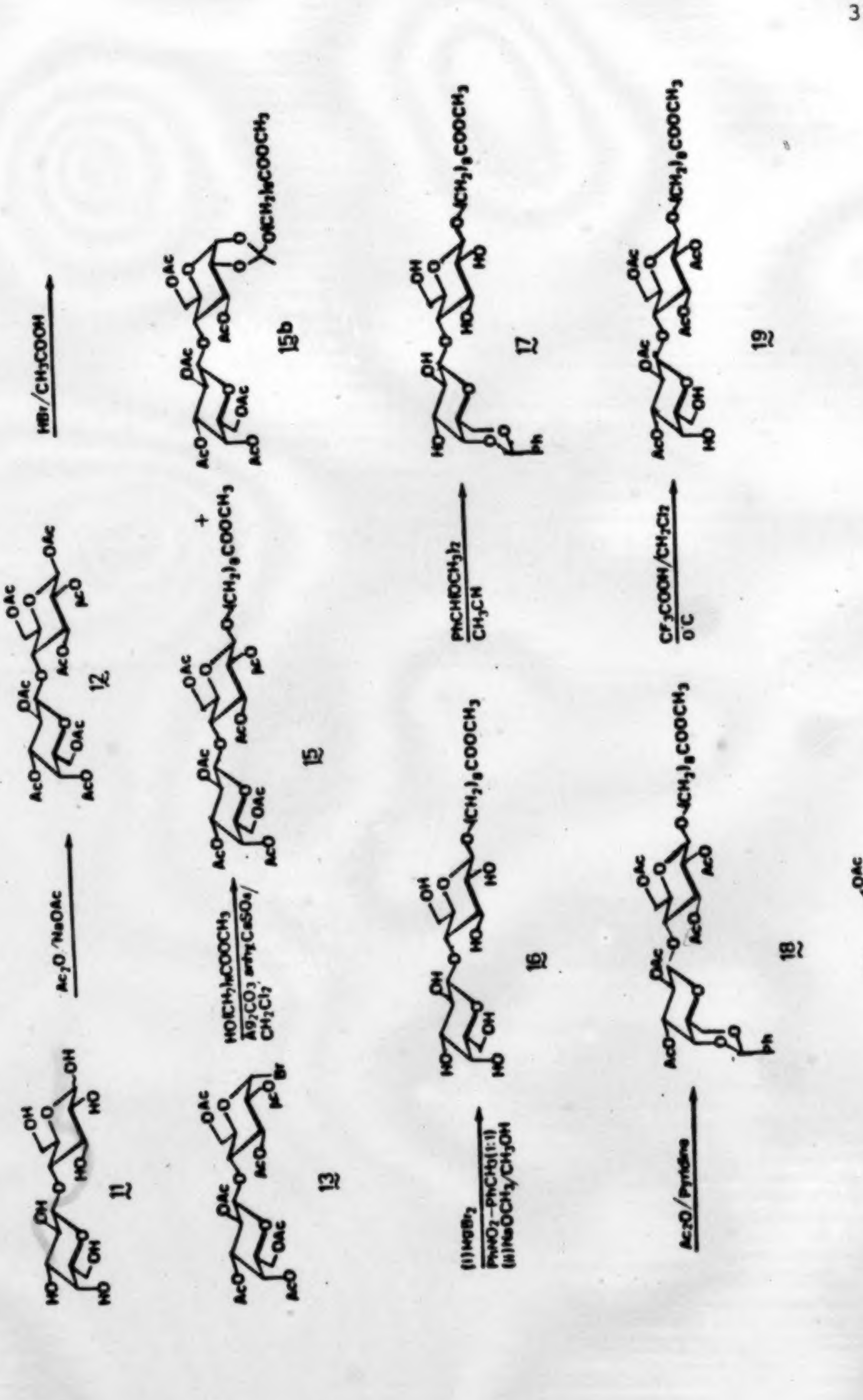
II. DISCUSSION OF RESULTS

A. Chemical Synthesis

As was mentioned in the Introduction, the goal of this investigation was to synthesize the tri- and tetrasaccharides known as *asialo*-G_{M2} and *asialo*-G_{M1} in the form of the haptens **8** and **9**. It is expected that these structures will prove to be of interest to immunochemical studies related to the G_{M2} and G_{M1} gangliosides. However, the main objective of this research program was to synthesize the structures and to study their conformational properties.

1. 8-Methoxycarbonyloctyl 2,3,6,2',3'-penta-*O*-acetyl-6'-*O*-benzoyl-β-D-lactoside (**20**)

Compound **20** was an intermediate for the synthesis of both *asialo*-G_{M2} (**8**) and *asialo*-G_{M1} (**9**) haptens. The synthesis started with commercial lactose (**11**) which was converted to the β-octaacetate (**12**) using a published procedure.[88] As was mentioned earlier, compound **12** was used by Banoub and Bundle[73] for the preparation of **15** in 60% yield by way of condensation with 8-methoxycarbonyloctanol (**14**) in the presence of stannic chloride. Attempts to prepare **15** in this manner failed. Instead of pursuing this approach further, it was decided to attempt the preparation by way of classical Koenigs-Knorr reaction[89] involving


11
Ac₂O/NaOAc
12
HBr/CH₃COOH
13
HO(CH₂)₈COOCH₃
Ag₂CO₃ anhy. CaSO₄/CH₂Cl₂
15
15b
(i) HgBr₂
PhNO₂–PhCH₃ (1:1)
(ii) NaOCH₃/CH₃OH
16
PhCH(OCH₃)₂
CH₃CN
17
Ac₂O/Pyridine
18
CF₃COOH/CH₂Cl₂
0°C
19
PhCOCl (1.1 eq)
Pyridine, −78°C,
CH₂Cl₂
20

Scheme 5

silver carbonate to promote the condensation of α-acetobromolactose (13) with the alcohol (14)*. The bromide 13 was prepared without difficulty from the octaacetate (12) in the usual way[90] and the condensation was performed in the presence of anhydrous calcium sulphate to absorb the water which is formed in the neutralization of the hydrogen bromide by the silver carbonate.

The Koenigs-Knorr reaction involving an acetobromo sugar is well known to often be accompanied by orthoester formation.[92] Indeed, the examination of the ^{1}H-nmr spectrum (Fig. 2a) of the product formed on reaction of 13 with 14 showed evidence for orthoester formation (15b). The signals at 1.7 ppm and 5.68 ppm are at positions typical for the orthoacetyl and H-1 of alkyl 1,2-orthoacetyl derivatives of glucose.[93] It is well known[94] that orthoesters can often be rearranged to the β-glycoside by treatment with Lewis acids such as mercuric bromide. Treatment of the crude product with mercuric bromide in toluene-nitrobenzene (1:1), in fact, caused the disappearance of the signals assigned to the orthoester. This result is evident from a comparison of the spectra in Fig. 2a and Fig. 2b. The spectrum of the

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*The alcohol 14 was a generous gift of Chembiomed Ltd., University of Alberta. For the preparation of 14, see reference 91.

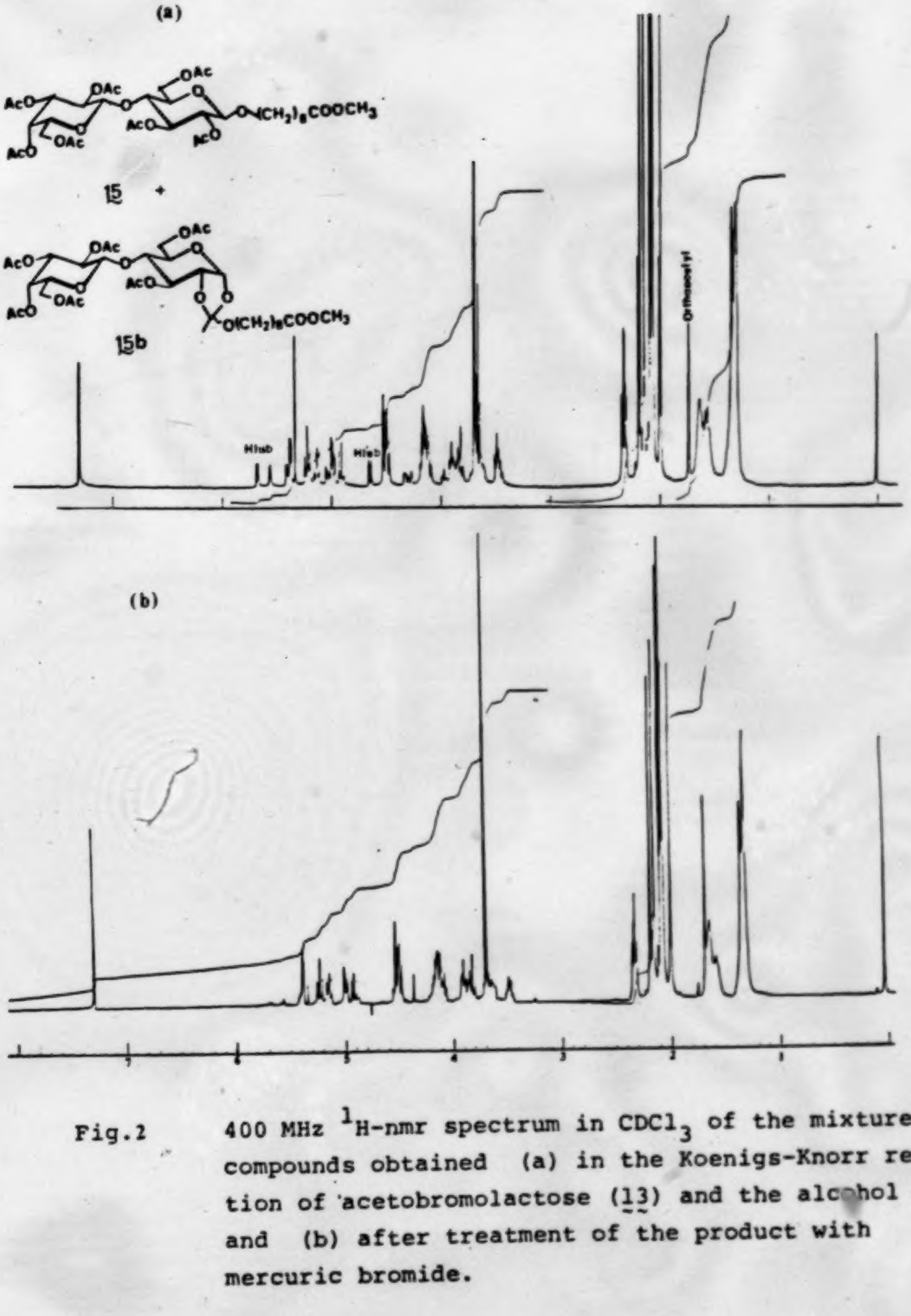


Fig.2 400 MHz ^{1}H-nmr spectrum in $CDCl_3$ of the mixture of compounds obtained (a) in the Koenigs-Knorr reaction of acetobromolactose (13) and the alcohol (14), and (b) after treatment of the product with mercuric bromide.

crude product is seen to be quite similar to that for the pure acetylated β-glycoside (**15**) Deacetylation of the crude product produced **16** which crystallized from methanol. The ^{1}H-nmr spectrum of this compound, reproduced in Fig. 4, clearly established its identity and high level of purity. Acetylation of **16** produced the authentic sample of the hepta-*O*-acetyl derivative (**15**) whose ^{1}H-nmr spectrum is reproduced in Fig. 3. The ^{13}C-nmr parameters for **15** were identical to those published by Banoub and Bundle.[73] Furthermore, a comparison of Fig. 2b with Fig. 3 shows that **15** was the major component of the crude product from the orthoester rearrangement. The yield of **16** based on **13** was 60% and this procedure avoided the use of chromatographic purification.

It is of interest to note that it was also attempted to prepare **15** by condensation of **13** with **14** under Helferich conditions which involved the use of mercuric cyanide as the promoter. This provided a mixture of α and β anomers, as was evident from the ^{1}H-nmr spectrum of the product formed on de-*O*-acetylation, which showed doublets at 4.94 ppm (J = 3.5 Hz) and 4.20 ppm (J = 7.8 Hz) (α:β = 1:1.6) and which are characteristic for α and β alkyl glucosides.[95] Separation of these anomers could not be achieved either by chromatography or by crystallization.

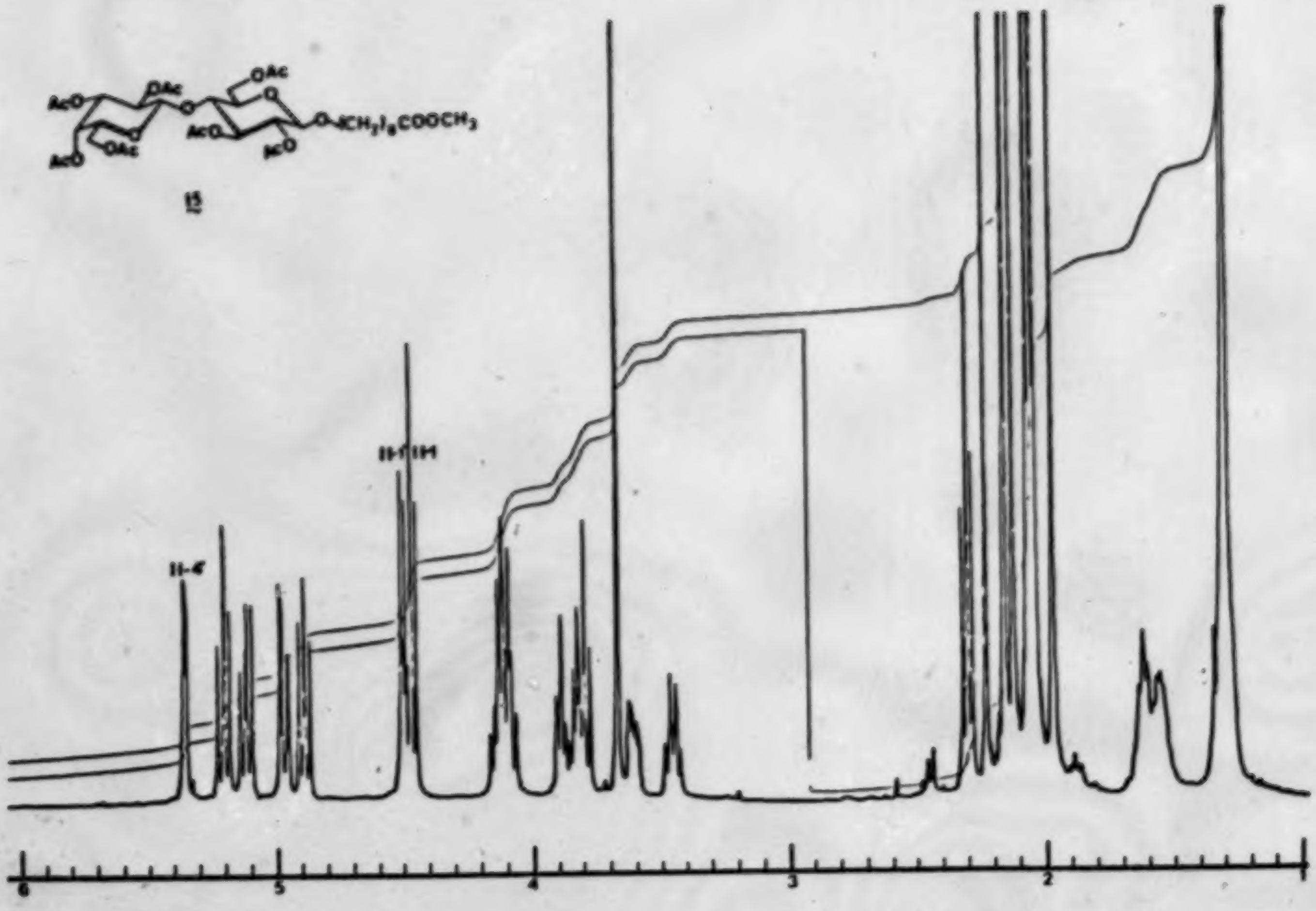


Fig.3   400 MHz ^{1}H-nmr spectrum of compound 15 in CDCl$_3$.

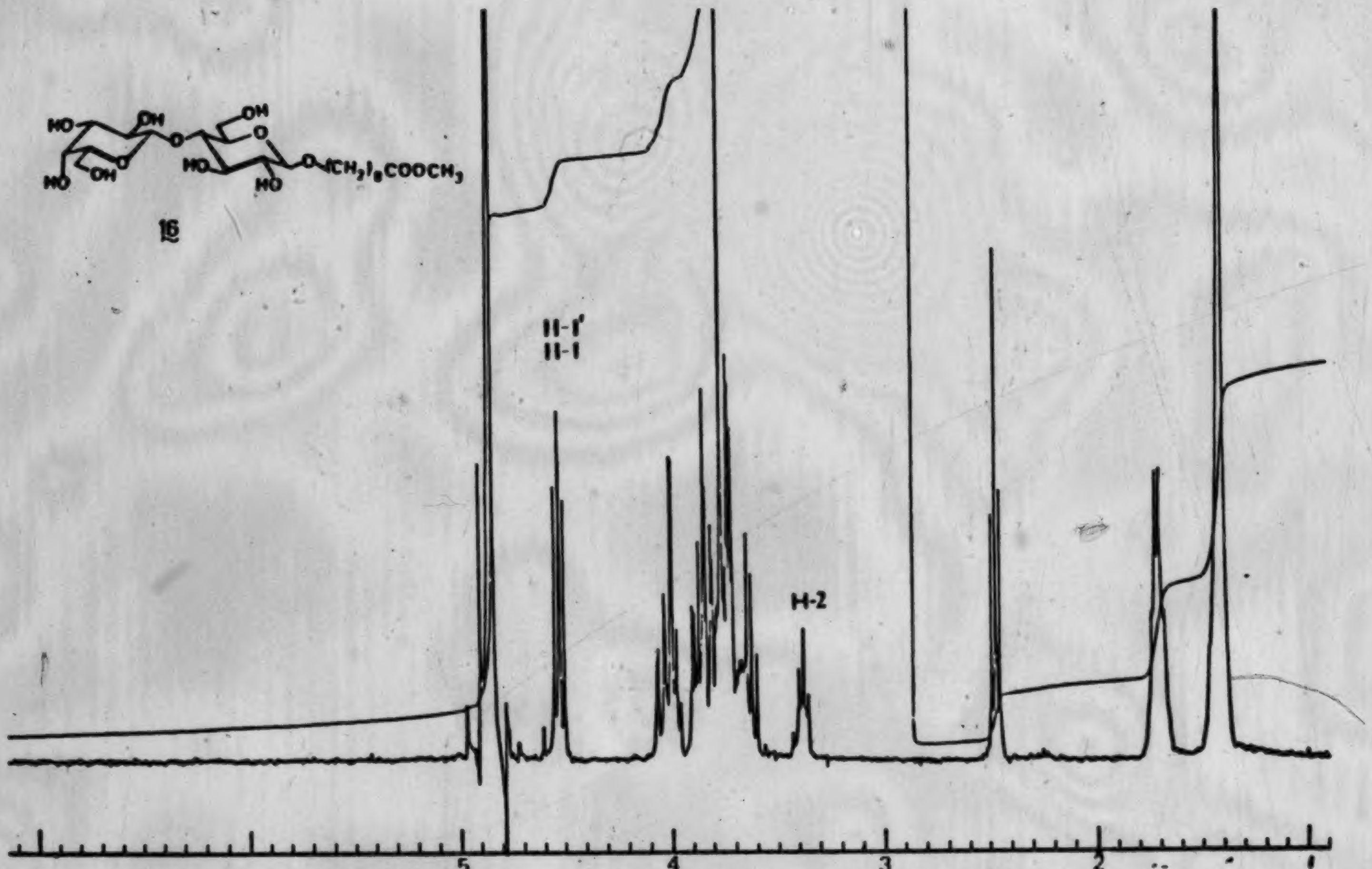


Fig.4 400 MHz ^{1}H-nmr spectrum of compound 16 in 100% deuterium oxide.

The first step in the conversion of the lactoside (16) to its appropriately blocked derivative (20) was to form the 4',6'-*O*-benzylidene derivative (17). This was readily accomplished under the conditions established by Evans[77] which employ a transacetalation between the alcohol and α,α-dimethoxytoluene. The product 17 was obtained as a colorless crystalline material in 78% yield when acetonitrile was used as a solvent. The use of *N*,*N*-dimethylformamide as solvent led to undesirable by-products which were not further investigated.

The ^{1}H-nmr spectrum of the benzylidene derivative 17 is reproduced in Fig. 5. The relative intensities of the signals require the presence of only one benzylidene group. It is well established that the signal in the ^{13}C-nmr spectra for the hydroxymethyl groups occur at 61±1 ppm.[96] In fact, the lactoside (16) showed signals at 61.27 and 60.45 ppm and the spectrum for 17 showed only one signal at this position of the ^{13}C-nmr spectrum. Therefore, one of the hydroxymethyl groups of 16 must have been involved in the formation of the benzylidene group of 17. That the hydroxymethyl group is that of the galactose unit was firmly established by a comparison of the ^{1}H-nmr spectra of the acetyl derivatives 15 and 18. It is well known that the equatorial H-4 of a galactose unit is very weakly coupled

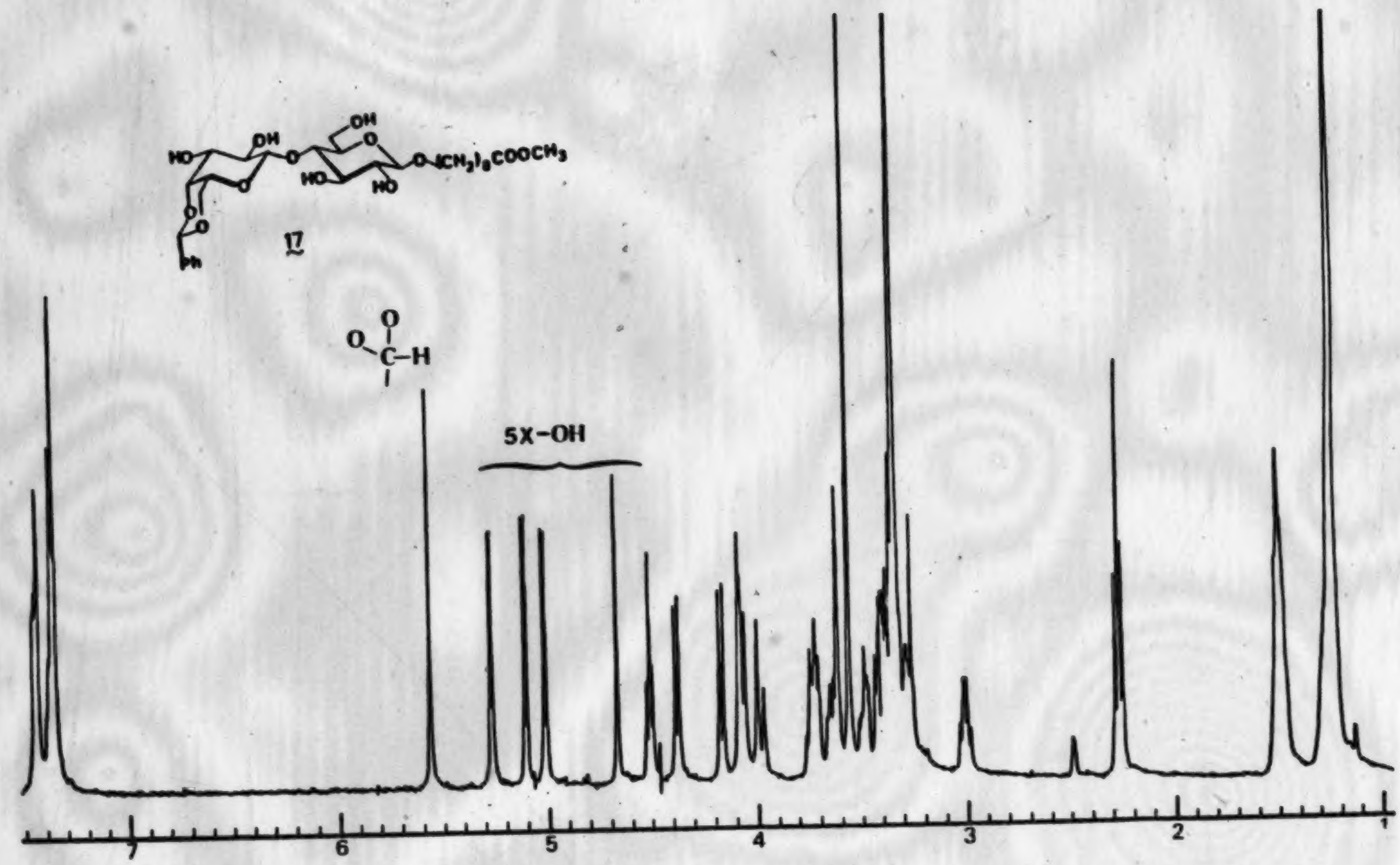
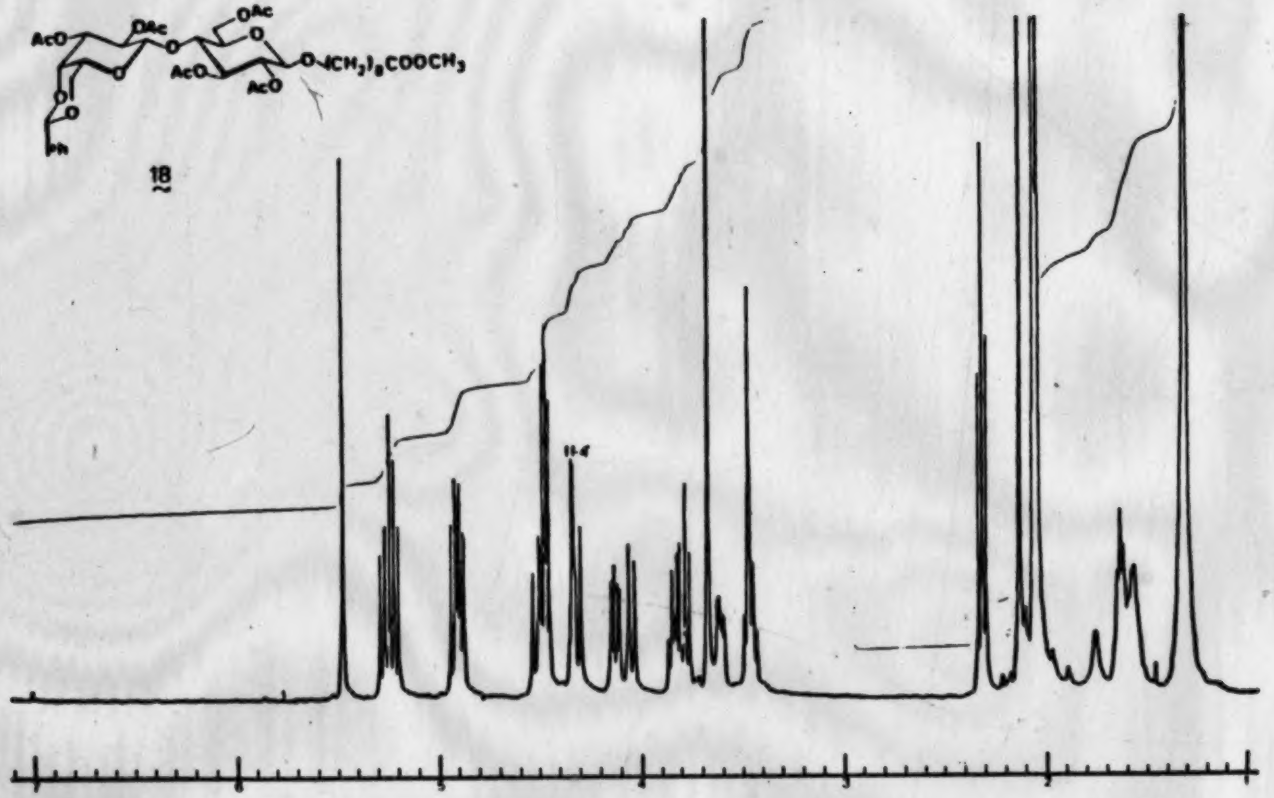


Fig. 5 400 MHz ^{1}H-nmr spectrum of compound 17 in DMSO-d_6.

to H-5. Thus, the assignment of the signal for H-4 is normally readily accomplished. As seen in Fig. 6, the signal for H-4' is at 5.34 ppm. In contrast, the signal for H-4' in the acetylated benzylidene lactoside (**18**) is at 4.32 ppm. Therefore, the 4'-position of the galactose unit of **18** must be involved in the benzylidene ring and, consequently, **18** must be a 4',6'-*O*-benzylidene derivative of **16**. The compound **18** was prepared by acetylation of **17** in 90% yield.

The preparation of **20** from **18** required first the removal of the benzylidene protecting group and the selective esterification of the resulting diol **19** at the primary position. Treatment of **18** with 90% aqueous trifluoroacetic acid[75] cleaved the acetal linkage of the 4',6'-*O*-benzylidene ring to provide the diol **19** in 75% yield. The ^{1}H-nmr spectrum of **19** is reproduced in Fig. 7. It is known that the coupling of the hydroxyl hydrogen to the vicinal protons can be observed provided the exchange of the hydroxyl hydrogen is slow compared to nmr time scale. In fact, for the diol **19**, the signals for the primary and secondary hydroxyl hydrogens were seen as a triplet and a doublet at 5.02 and 5.12 ppm, respectively, which indicated that no acetyl migration had taken place from the other *O*-acetyl groups to the 6'-hydroxyl group. The coupling of



18

Fig.6 400 MHz ^{1}H-nmr spectrum of compound 18 in $CDCl_3$.

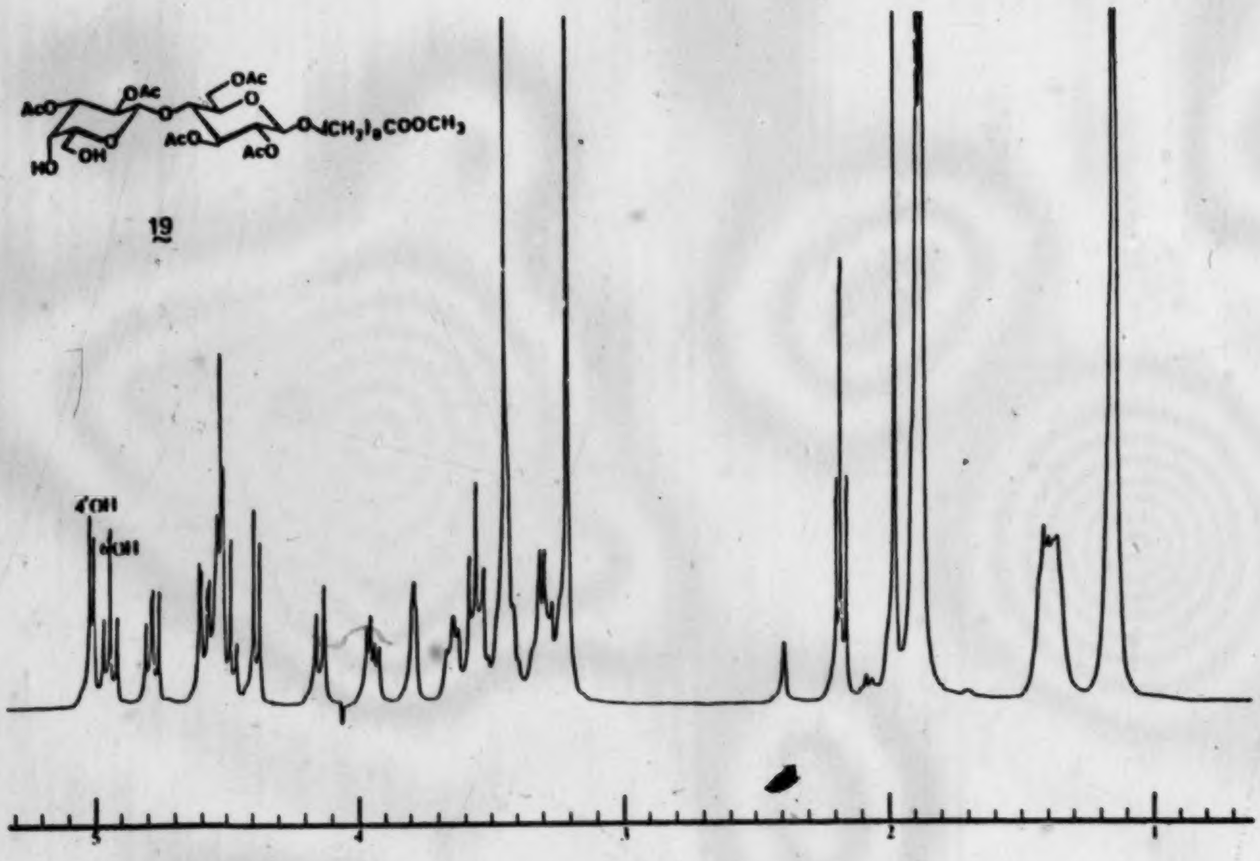


Fig.7 360 MHz ^{1}H-nmr spectrum of compound 19 in DMSO-d_6.

4'-hydroxyl hydrogen with H-4' caused the signal due to this hydrogen to appear as a broad triplet, in contrast to the normal broad doublet for this proton. Furthermore, as expected, the ^{13}C-nmr spectrum showed two signals at 62.47 and 61.41 ppm, in contrast to only one signal observed for **18** around 62 ppm. Thus, the ^{1}H and ^{13}C chemical shifts of the atoms firmly established the assigned structure **19** for the diol.

The selective protection of the primary hydroxyl group of the diol (**19**) was achieved in greater than 80% yield with 1.1 equivalents of benzoyl chloride and pyridine under mild conditions (-78 to -20°C) which provided the desired alcohol **20** as an amorphous material. The ^{1}H-nmr spectrum of **20** (Fig. 8) indicated the alcohol **20** to be essentially pure. The relative intensities of the aromatic hydrogens require the presence of only one benzoate grouping in **20**. The chemical shift of H-4' is 3.98 ppm and this established beyond any doubt the absence of ester grouping at this position. Furthermore, by decoupling experiments, the signals for H-6'a and H-6'b were identified between 4.36 and 4.26 ppm, which required the presence of the ester group at this position. The appearance of hydrogens at 6'-position at a lower field compared to the acetates, as seen in acetylated lactoside **15**, clearly established the presence of stronger electron withdrawing benzoate group at this position.

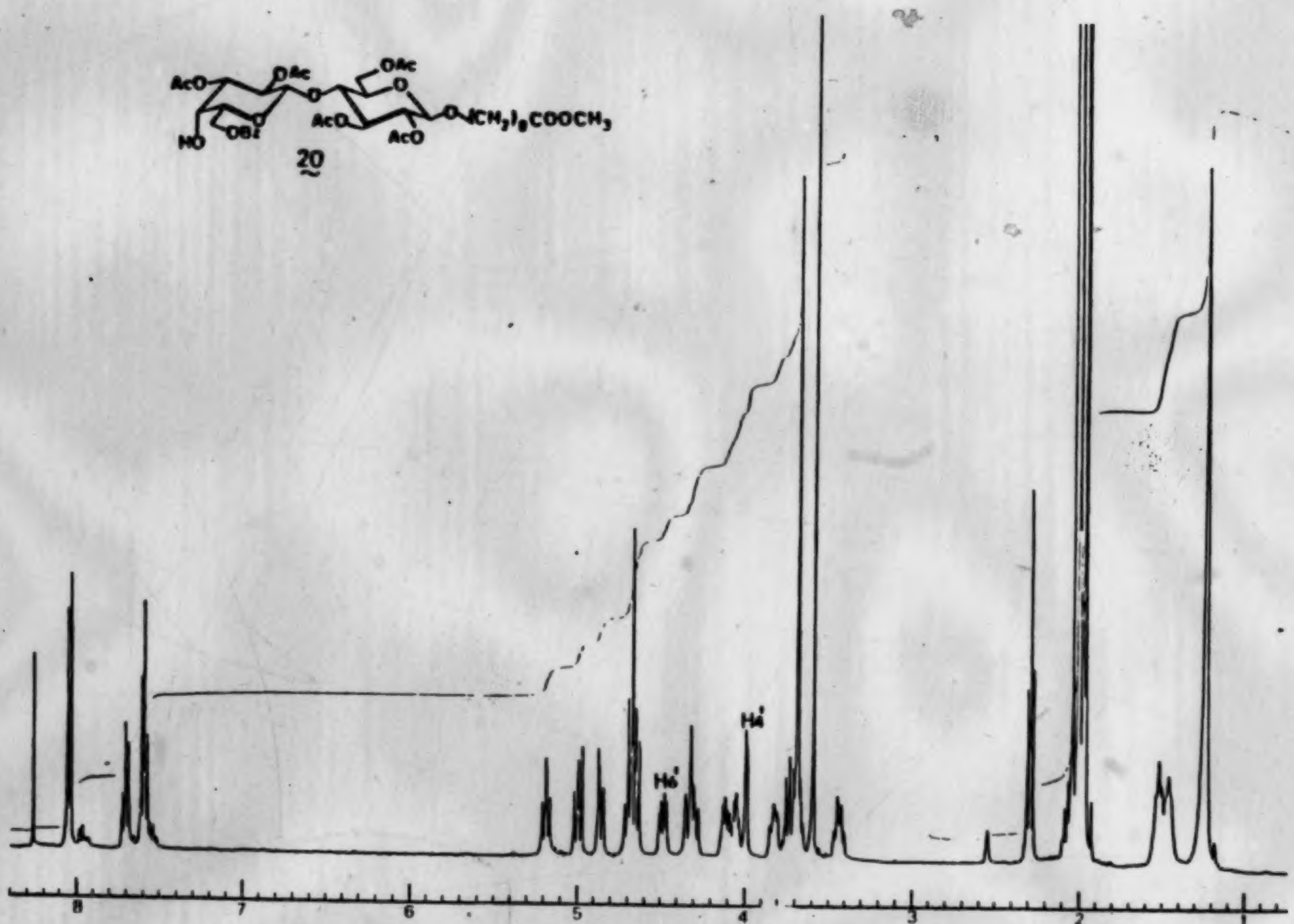


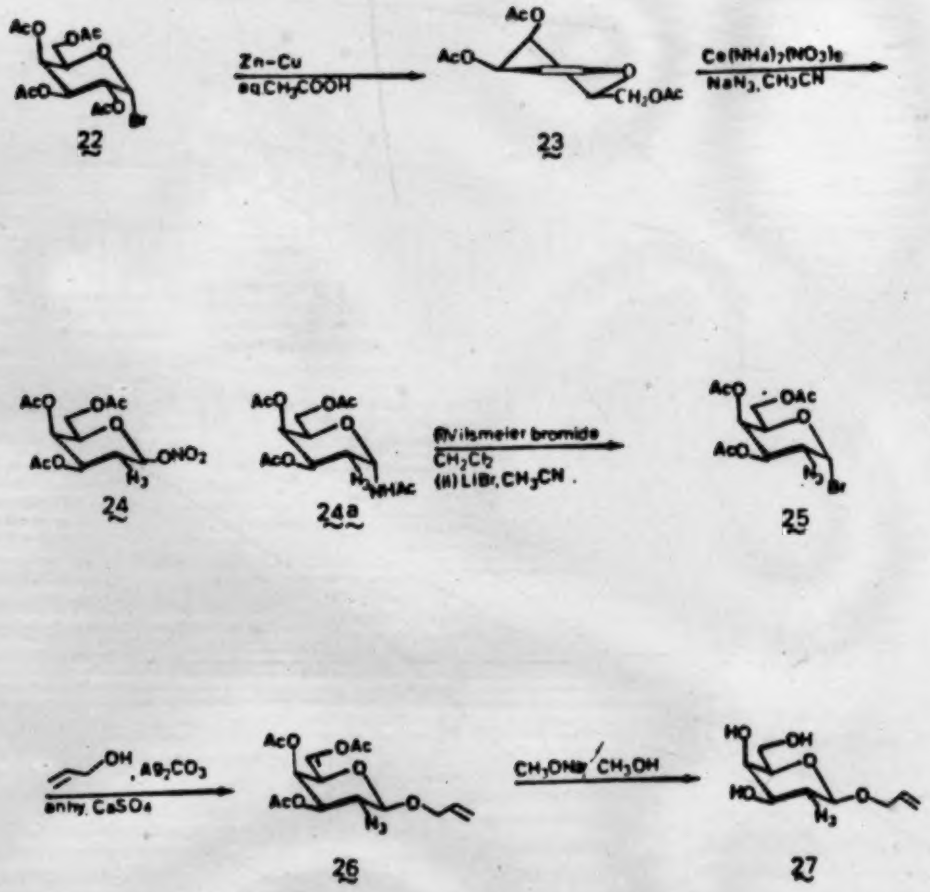
Fig.8 400 MHz ^{1}H-nmr spectrum of compound 20 in DMSO-d$_6$.

Thus, the desired alcohol intermediate (20) was prepared in large quantities from the readily available commercial lactose. This reaction sequence involves eight steps and, except in the last two steps, the use of chromatographic purification was avoided. The next task was, therefore, to prepare appropriately blocked phthalimido-bromides for the condensation with the alcohol 20 in order to accomplish the synthesis of *asialo*-G_{M2} (8) and *asialo*-G_{M1} (9) haptens.

2. Allyl 2-azido-2-deoxy-β-D-galactopyranoside (27)

The allyl glycoside (27) was a common intermediate for the preparation of suitably protected phthalimido-bromides required for the synthesis of blocked derivatives of *asialo*-G_{M2} and *asialo*-G_{M1} haptens. It was expected that 27 could be readily prepared via the azido bromide (25) as outlined in Scheme 6.

Lemieux and Ratcliffe[67] have reported the preparation of 25 from the mixture of azidonitrates 24 obtained in the azidonitration of tri-*O*-acetyl-D-galactal (23). However, it was found that the purity of the acetylated galactal (23) obtained by a published procedure which uses a copper-platinum couple[97] to reduce α-acetobromogalactose[101] left


AcO
OAc
AcO
AcO
Br
22
Zn–Cu
aq CH3COOH
AcO
AcO
CH2OAc
23
Ce(NH4)2(NO3)6
NaN3, CH3CN
AcO
OAc
AcO
N3
ONO2
24
AcO
OAc
AcO
N3
NHAc
24a
(i) Vilsmeier bromide
CH2Cl2
(ii) LiBr, CH3CN.
AcO
OAc
AcO
N3
Br
25
OH, Ag2CO3
anhy. CaSO4
AcO
OAc
AcO
N3
26
CH3ONa/CH3OH
HO
OH
HO
N3
27

Scheme 6

much to be desired. Therefore, an investigation to improve the quality and the yield of **23** was undertaken. Even though the ^{1}H-nmr spectrum of the crude product (Fig. 9a) indicated that the major product was the desired tri-*O*-acetyl-D-galactal (**23**), it was found to be contaminated up to 30% with undesired side products, as was estimated from the relative intensities of the signal at 6.3 ppm due to H-1 and the acetyl signals around 2 ppm. Therefore, the distillation of the crude product was attempted without much success in significantly improving the purity. Since it had been reported earlier by Roth and Pigman[98] that the use of the less expensive zinc-copper couple under buffered conditions effected the transformation of α-acetobromoglucose to the acetylated glucal in good yield and high purity, the use of this reagent to transform α-acetobromogalactose (**22**) to **23** was examined. In fact, when the procedure was repeated, it was found that the product (**23**) was formed in greater than 90% yield and was more than 90% pure (as indicated by the ^{1}H-nmr spectrum, Fig. 9b). This point is illustrated in Figs. 9b and 9c where the ^{1}H-nmr

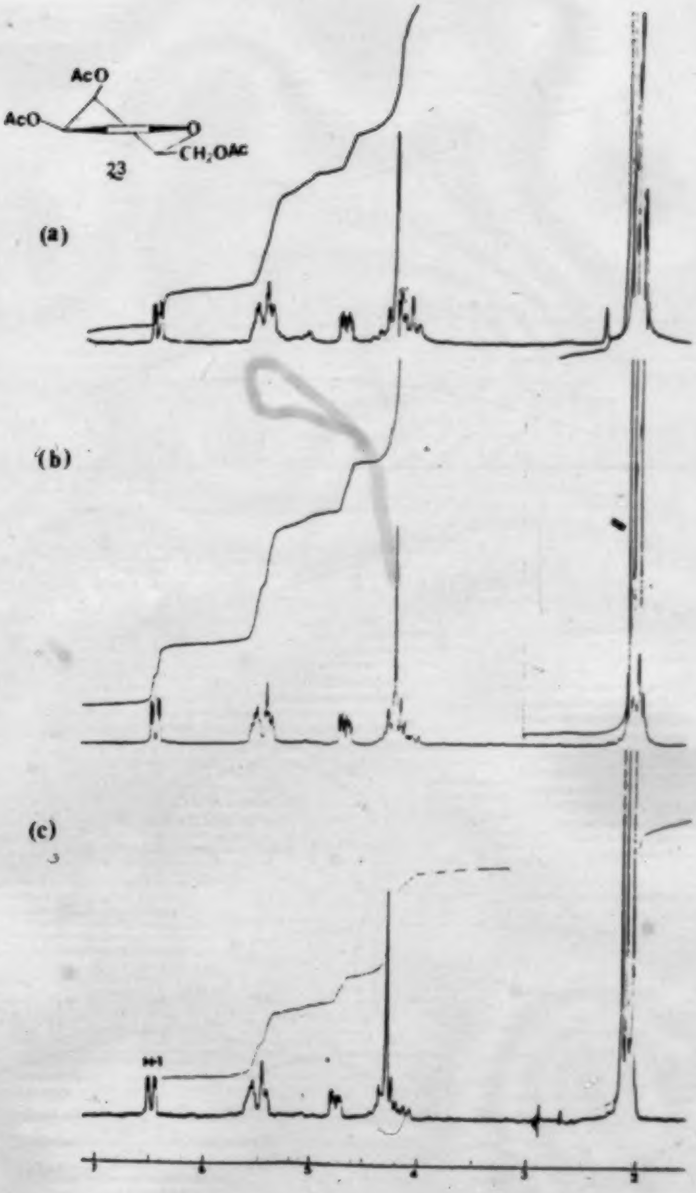


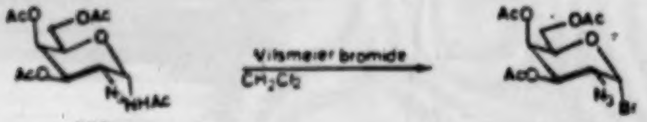
Fig. 9 100 MHz ^{1}H-nmr spectra of compound 23 in $CDCl_3$. a. the crude compound 23 obtained from zinc-platinum couple. b. the crude compound 23 obtained from zinc-copper couple. c. the distilled compound 23 of zinc-copper couple.

spectrum of the crude preparation of 23 obtained using the zinc-copper couple and that obtained for the material after careful distillation are reproduced. As can be seen from these figures, the crude product was as pure as the distilled material and therefore the crude 23 was subsequently employed in the azidonitration reaction.

The azidonitration reaction was performed according to the procedure described by Lemieux and Ratcliffe[67] which involved the reaction between 23 and ceric ammonium nitrate-sodium azide (2:1) complex to provide an anomeric mixture of 2-azido-1-nitrates 24 and *N*-acetyl-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-*D*-galactopyranosylamine (24a). The ratio of products 24 and 24a and the yield were in accordance with the reported values.[67] Purification at this stage by chromatography was necessary in order to remove the undesired product 24a from the nitrates (24). Treatment of the nitrate (24) with lithium bromide and chromatographic purification then provided the desired azidobromide 25 (60% yield based on the nitrates).

In order for the azidonitration procedure to become more attractive to the large-scale preparation of 25, it was necessary also to convert the undesired 24a to the azido bromide (25). It was hoped that besides improving the yield, this improvement in procedure would avoid the chromatographic purification of azidonitration products. Thus, the hydrolysis of the labile nitrates on silica gel could be prevented.

In this regard, it was found that the treatment of **24a** with *N*,*N*-dimethylbromoforminium bromide (Vilsmeier bromide) converted it to the desired azidobromide (**25**) in near quantitative yield, as indicated by the ^{1}H-nmr spectrum (Fig. 10b) of the crude reaction product. Consequently, the crude



24a   **25**

azidonitration reaction product was first reacted with Vilsmeier bromide to effect the conversion of **24a** to **25** and then with anhydrous lithium bromide to transform the nitrates (**24**) to **25**. After chromatographic purification, the overall yield of the bromide (**25**) was improved to 40% based on acetobromogalactose (**22**) in contrast to 16% yield obtained by the procedure which involved the distillation of **23** and chromatographic purification of azido-nitrates.[67] The nmr parameters and the physical constants of the bromide (**25**) were

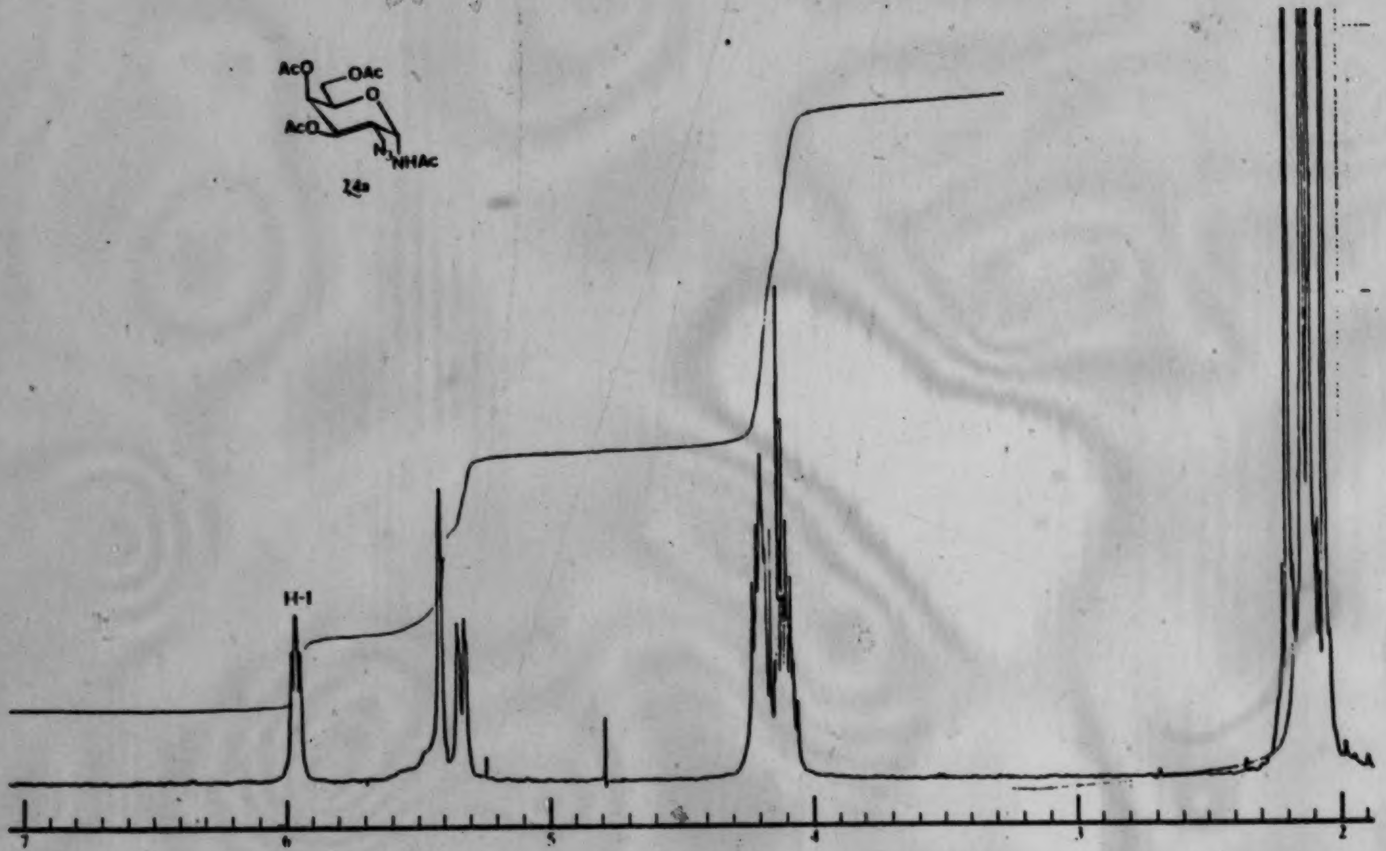


Fig. 10a 400 MHz ^{1}H-nmr spectrum of compound 24a in $CDCl_3$.

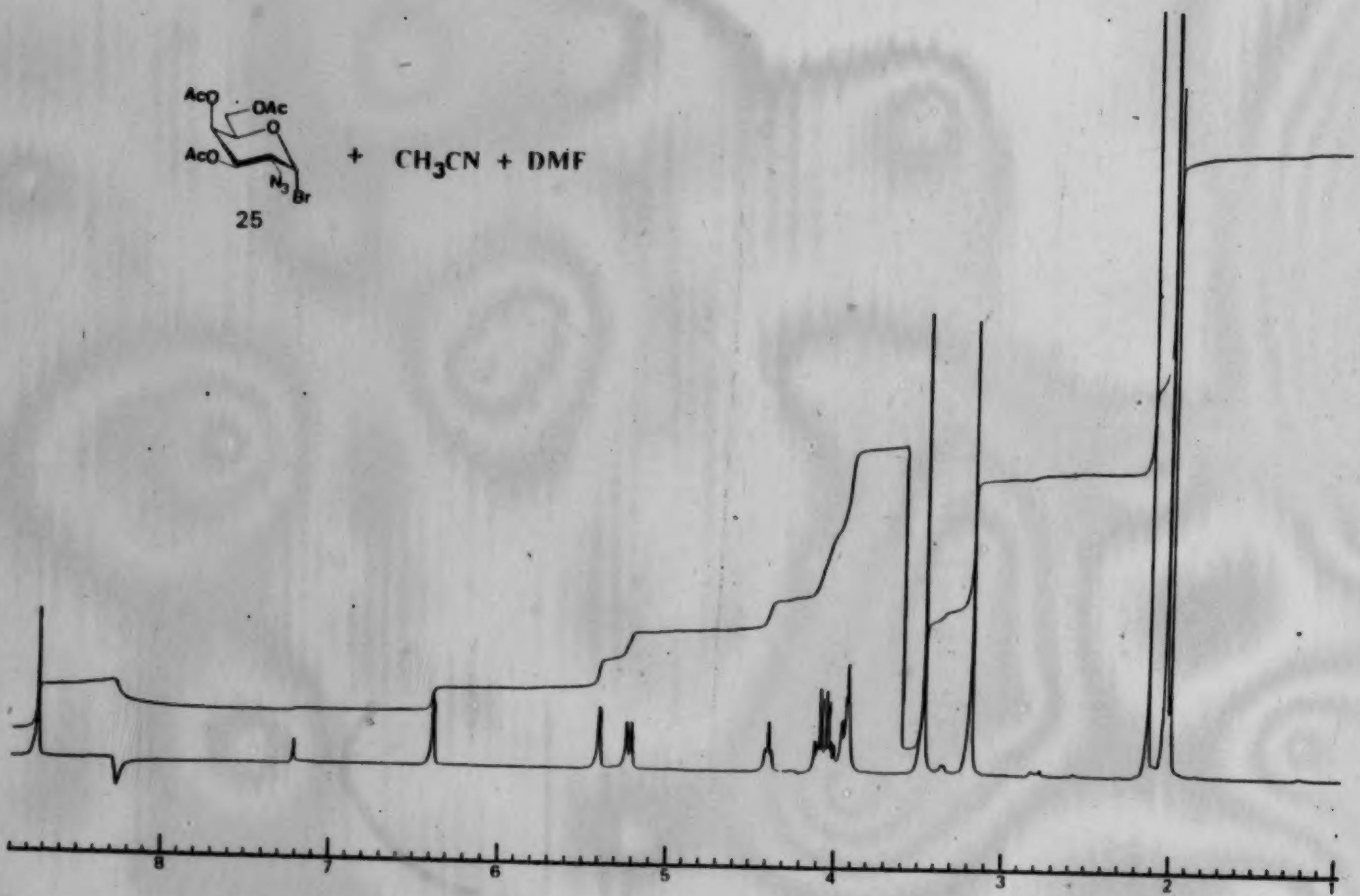


Fig.10b 360 MHz ^{1}H-nmr spectrum of the reaction mixture obtained by the treatment of compound 24a with Vismeier bromide, in $CDCl_3$.

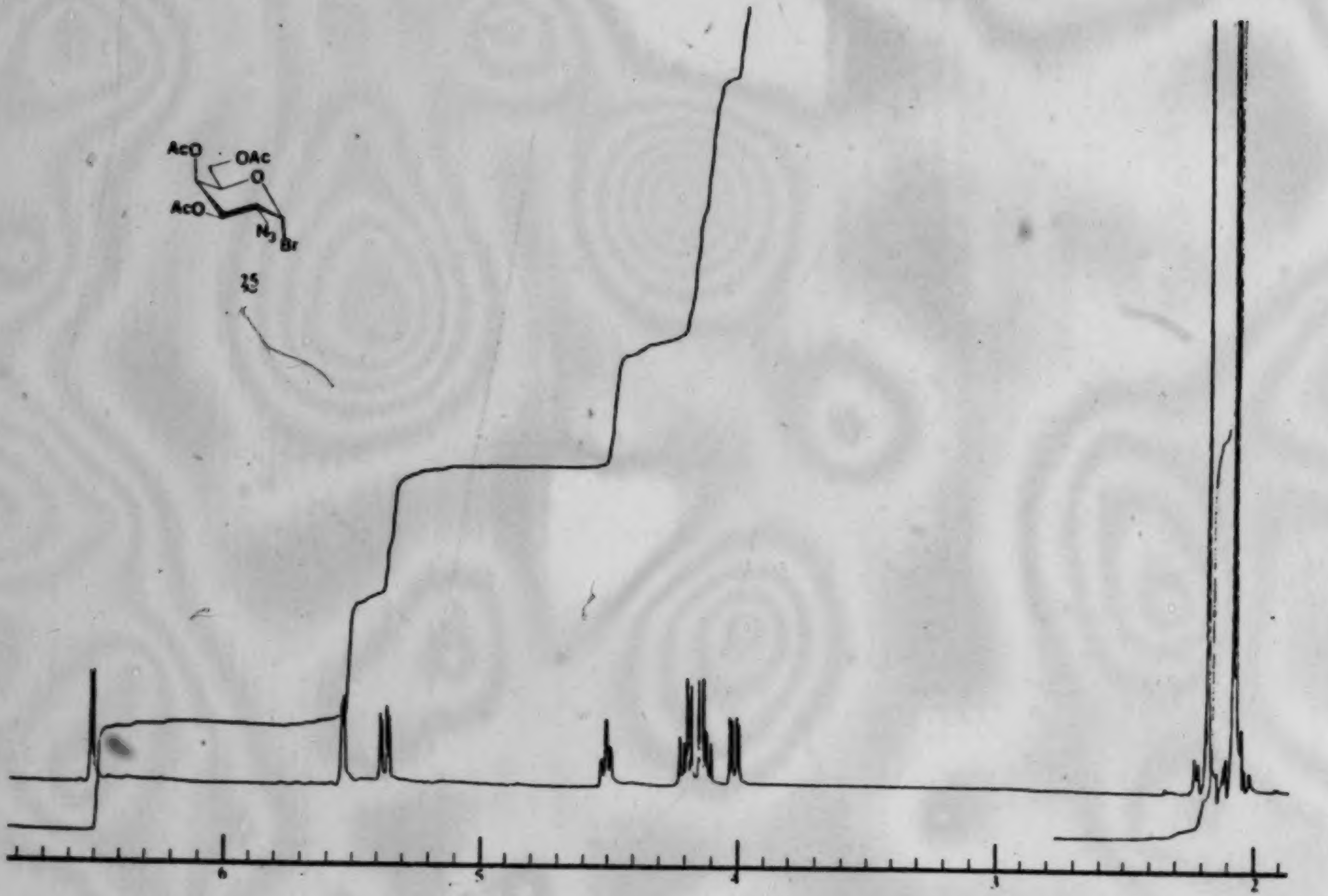


Fig. 10c 400 MHz ^{1}H-nmr spectrum of compound 25 in $CDCl_3$.

identical to those reported by Lemieux and Ratcliffe.[67]

The ^{1}H-nmr spectrum of the bromide (25) after chromatographic purification is reproduced in Fig. 10c. Comparison of relative intensities of signals of the crude product with pure bromide indicated the former to be more than 70% pure. Consequently, the chromatographic purification of the bromide was avoided in an attempt to employ the crude product directly in a Koenigs-Knorr reaction with allyl alcohol.

The allyl 2-azido-2-deoxy-glycoside 26 was prepared from the crude azido-bromide 25 and allyl alcohol by the classical Koenigs-Knorr reaction which involved the use of silver carbonate as the promoter. Anhydrous calcium sulphate was included in the reaction mixture to absorb the water produced in the reaction as a result of neutralization of the liberated hydrogen bromide by silver carbonate. After chromatographic purification, 26 was obtained as a syrup in 29% overall yield based on acetobromogalactose (22) (59% based on crude bromide). On the other hand, by employing pure glycosyl bromide 25, the allyl glycoside 26 could be obtained in 75% yield. The ^{1}H- and ^{13}C-nmr spectra of this product (Fig. 11) clearly established its identity and a high level of purity. The chemical shift (4.4 ppm) and the coupling constant (J = 7.8 Hz) observed for the anomeric hydrogen required the trans-diaxial relationship

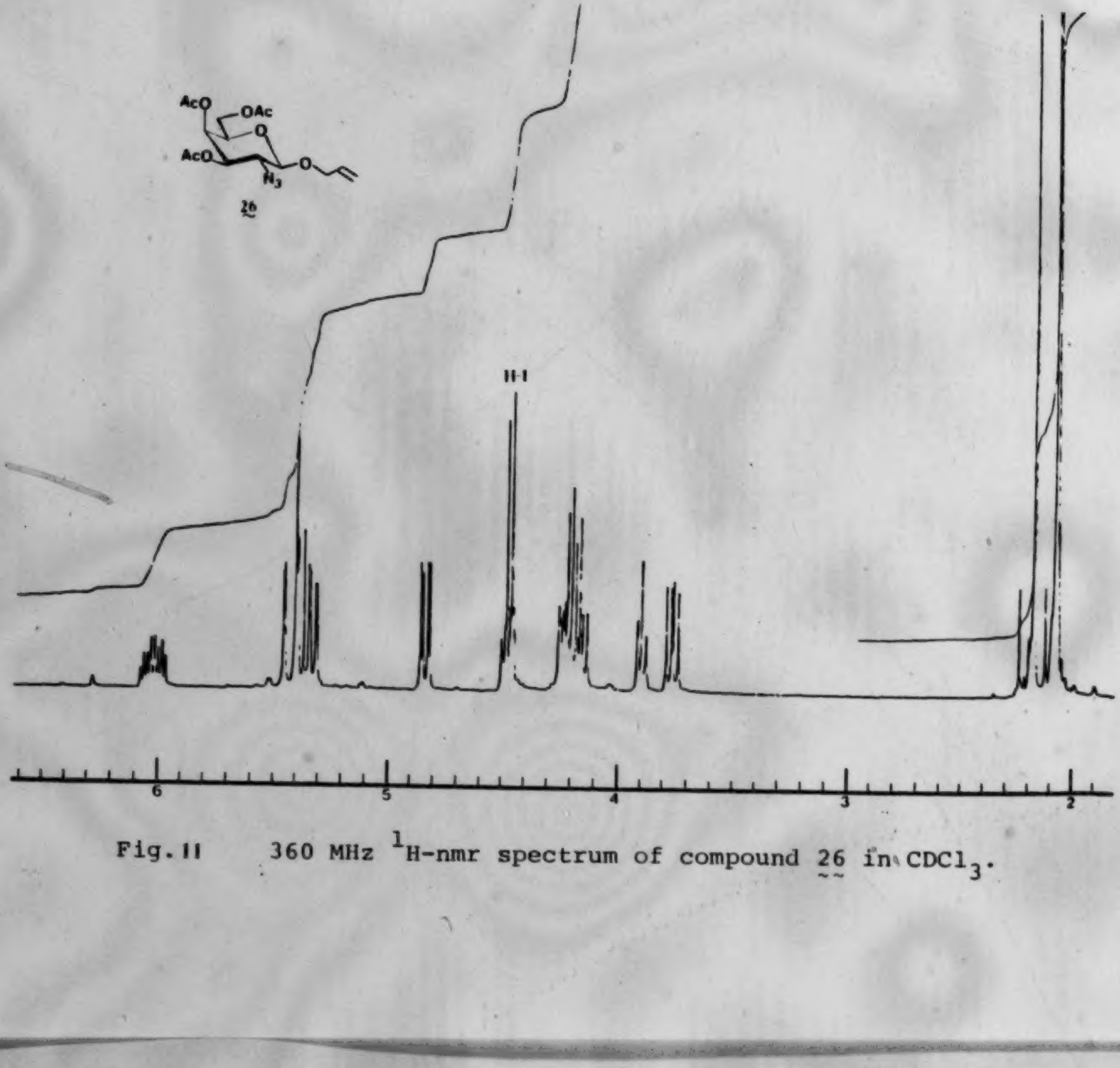


Fig. 11 360 MHz ^{1}H-nmr spectrum of compound 26 in CDCl$_3$.

of H-1 and H-2 and consequently confirms the β-configuration at the anomeric center. As expected, the signal for H-4 appeared at the lowest field (5.4 ppm) compared to other ring hydrogens.

Finally, the de-*O*-acetylation of **26** under Zemplen's conditions using sodium methoxide in methanol provided the desired allyl glycoside (**27**) in near quantitative yield. Crystallization from ethyl acetate-n-hexane provided **27** as a colorless crystalline solid which was indicated to be highly pure by ^{1}H-nmr (Fig. 12). The chemical shifts and coupling constants established beyond any doubt the assigned structure.

3. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide (**32**)

The title compound **32** was required for the condensation with the alcohol **20** to accomplish the synthesis of the blocked derivative of *asialo*-G_{M2} (**33**). As mentioned earlier, rather than to attempt its synthesis from D-galactosamine, the compound was prepared from **27** as outlined in Scheme 7.

It seemed desirable to prepare the phthalimido glycoside **29** from **27** rather than from the acetate **26** since the intermediate *O*-acetylated amine could give rise to *O* to *N*-acetyl migration with the formation of an acetamido group.

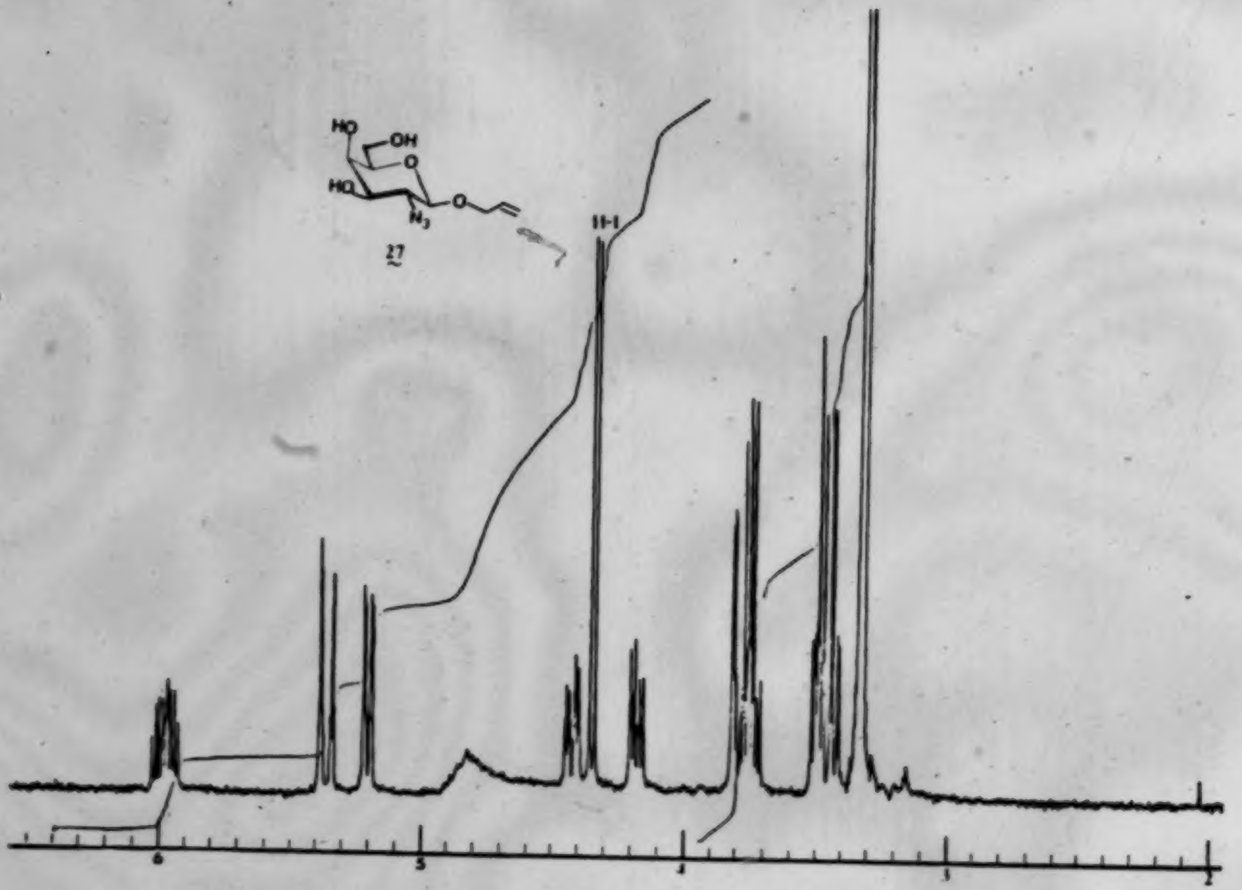
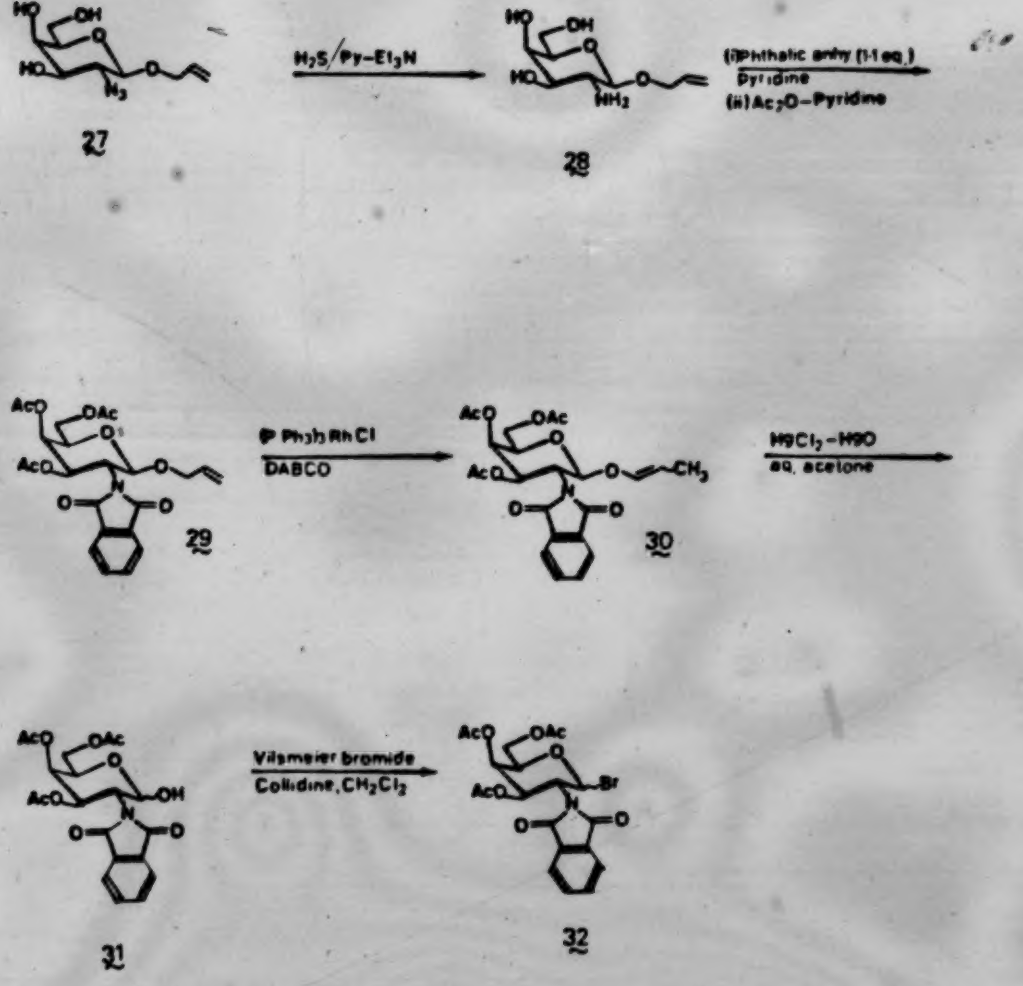


Fig.12 400 MHz ^{1}H-nmr spectrum of compound 27 in CD_3OD.


27
H2S/Py-Et3N
28
(i)Phthalic anhy (1.1 eq.)
Pyridine
(ii) Ac2O-Pyridine
29
(Ph3P)3RhCl
DABCO
30
HgCl2-HgO
aq. acetone
31
Vilsmeier bromide
Collidine, CH2Cl2
32

Scheme 7

The reduction of the azide (**27**) to the amine (**28**) was carried out with hydrogen sulphide in pyridine-triethylamine according to the conditions described by Lemieux and co-workers.[70] The crude product obtained seemed to be the desired amine as indicated by its low mobility on tlc and no attempt was made to further characterize this intermediate. Instead, the crude amine (**28**) was converted to the phthalimido compound (**29**) using published procedures.[70] This involved first reacting the amino group with phthalic anhydride to form the *N*-2-carboxybenzamide derivative followed by acetylation with pyridine and acetic anhydride to effect the ring closure as well as *O*-acetylation. After chromatographic purification, the desired phthalimido glycoside (**29**) was obtained as an amorphous material in 67% yield based on **27**.

The ^{1}H-nmr spectrum of **29** is reproduced in Fig. 13. The relative intensities of the aromatic signal require the presence of only one benzene ring in the molecule. The presence of the phthalimido group was expected[61] to cause a severe non-bonded deshielding interaction between the carbonyl group of the phthalimido function[53] and the axial H-1 and H-3 atoms. In fact, it can be seen from Figure 13 that both H-1 and H-3 are deshielded by 0.9 ppm compared to their chemical shifts in **26**. The magnitude

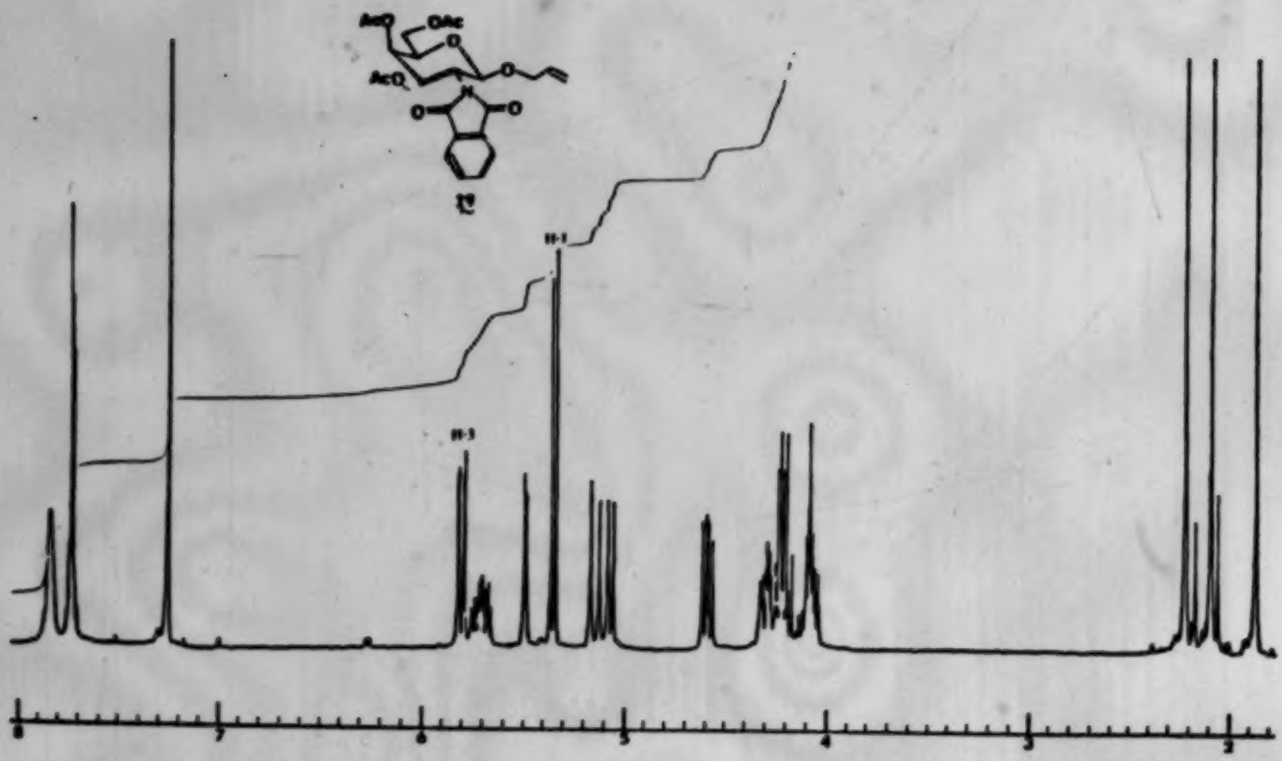


Fig. 13 400 MHz ^{1}H-nmr spectrum of compound 29 in $CDCl_3$.

of deshielding is so large that H-3 appears at even lower field than do the olefinic hydrogens in the aglycon. In the case of the ^{13}C-nmr spectrum of 29, a shielding of 2 ppm was observed for both C-1 and C-3 as a result of the introduction of the phthalimido group at the 2-position.

As expected, the allyl deprotection at the anomeric center could be readily accomplished under the conditions described by Gigg and Gent.[69] This involved the isomerization of the O-allyl to the O-propenyl group and the hydrolysis of the labile propenyl ether with an acidic reagent.[69] The isomerization of the allyl group was carried out by treating 29 with a catalytic quantity of tris-triphenylphosphine-rhodium(III)chloride in the presence of a base, normally 1,4-diazabicyclooctane, under refluxing conditions. The progress of the reaction was conveniently followed by ^{1}H-nmr examination of the reaction mixture (Fig. 14). After 24 hours, the major component in the reaction mixture was found to be the 1-propenyl glycoside (30) (multiplets at 6.3 and 1.6 ppm due to H-1 and the hydrogens of the olefinic methyl). The 1-propenyl glycoside 30 was then hydrolyzed with mercuric chloride-mercuric oxide in aqueous acetone to provide 31 in 80% yield.

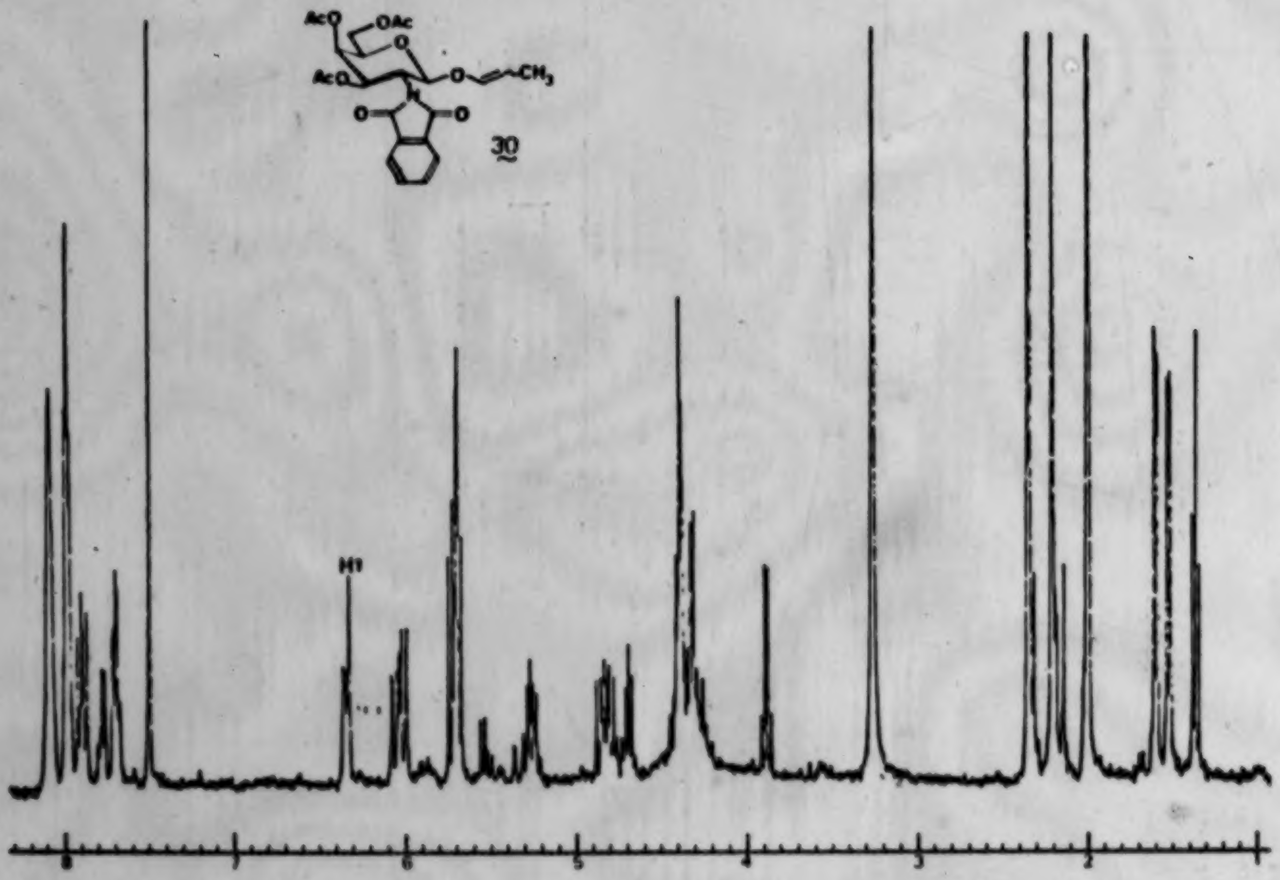


Fig.14 400 MHz ^{1}H-nmr spectrum of the reaction mixture containing compound 30, tris-triphenylphosphine rhodium(III)chloride and 1,4-diazabieyelooctane.

The ^{1}H-nmr spectrum (Fig. 15) indicated that the compound was an essentially pure mixture of the α and β anomers in the ratio 3:7. The quartets at 6.4 and 5.9 ppm, each with spacings of 3.5 and 7.5 Hz, are assigned to the H-3 proton of 31α and 31β, respectively. The low field signal is assigned to H-3 of the α-anomer since it is in *syn*-diaxial orientation with the hydroxyl group at C-1 and, consequently, it is expected to be at a lower field than the signal for H-3β. It is interesting to note that the anomeric hydrogens of the anomers have the same chemical shift (5.5 ppm) in contrast to the larger chemical shift value (lower field) normally observed for the anomeric hydrogens of α as compared to β-galactosides.[95] However, this is not surprising since, as mentioned earlier, the anomeric hydrogen of the β-anomer is expected[61] to be strongly deshielded by the phthalimido carbonyl group.

Finally, the desired phthalimido-bromide (32) required for the glycosylation of the alcohol (20) was prepared by reacting the 1-hydroxy compound with Vilsmeier bromide.[70] This provided a mixture of anomeric bromides in greater than 90% yield as an amorphous material. Recrystallization from ethyl acetate-n-hexane provided the β-bromide 32 which was highly pure as indicated by the ^{1}H-nmr spectrum (Fig. 16). But, for the purpose of glycosylation

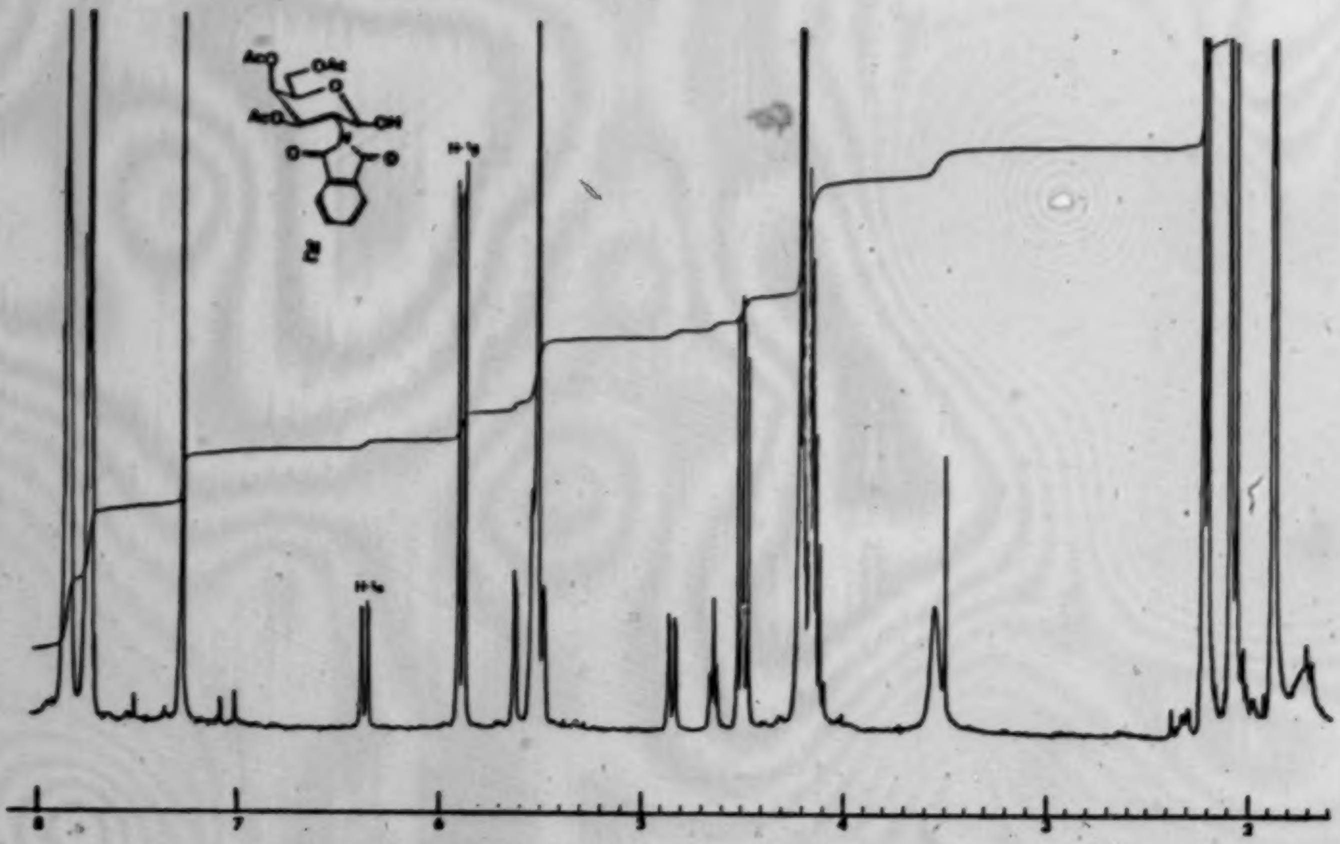


Fig.15 400 MHz ^{1}H-nmr spectrum of anomeric mixture of compound 31.

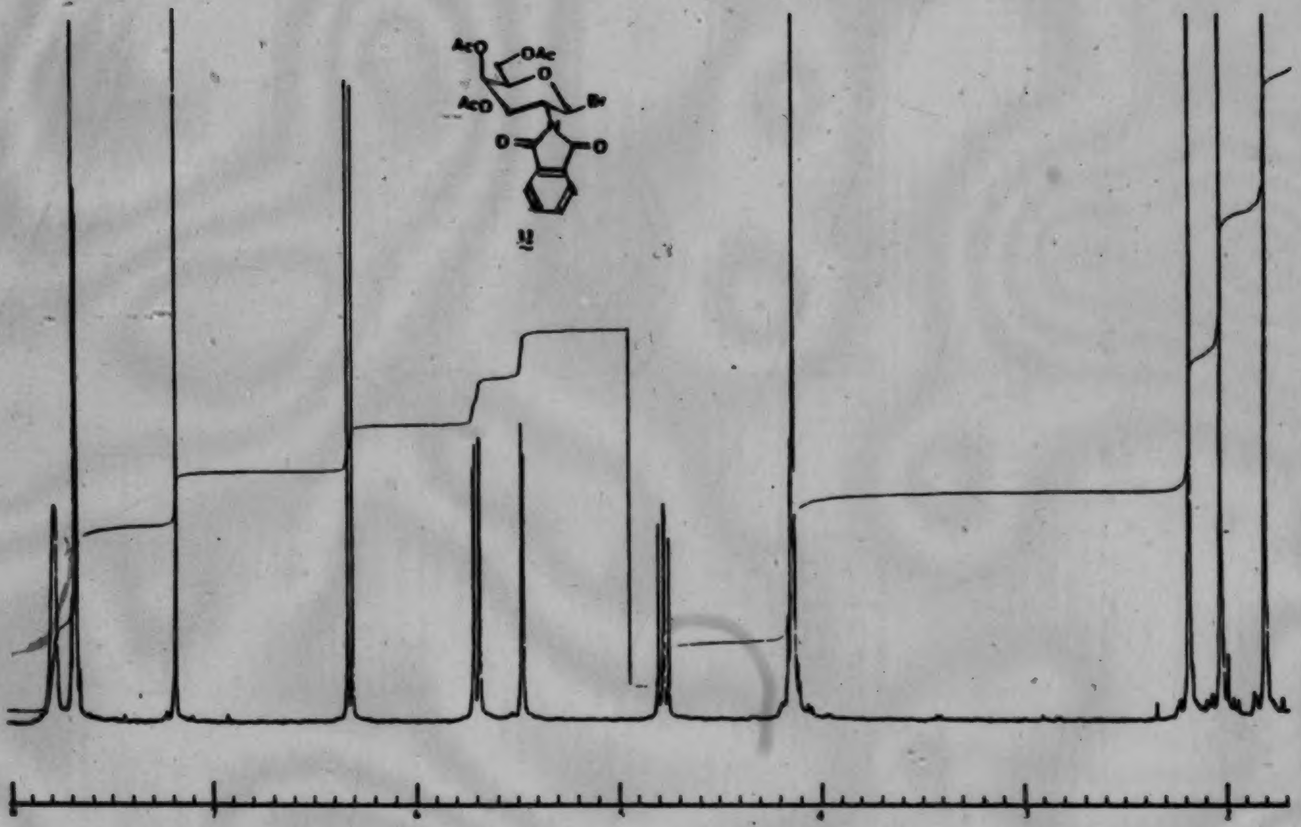


Fig.16 400 MHz ^{1}H-nmr spectrum of compound 32 in $CDCl_3$.

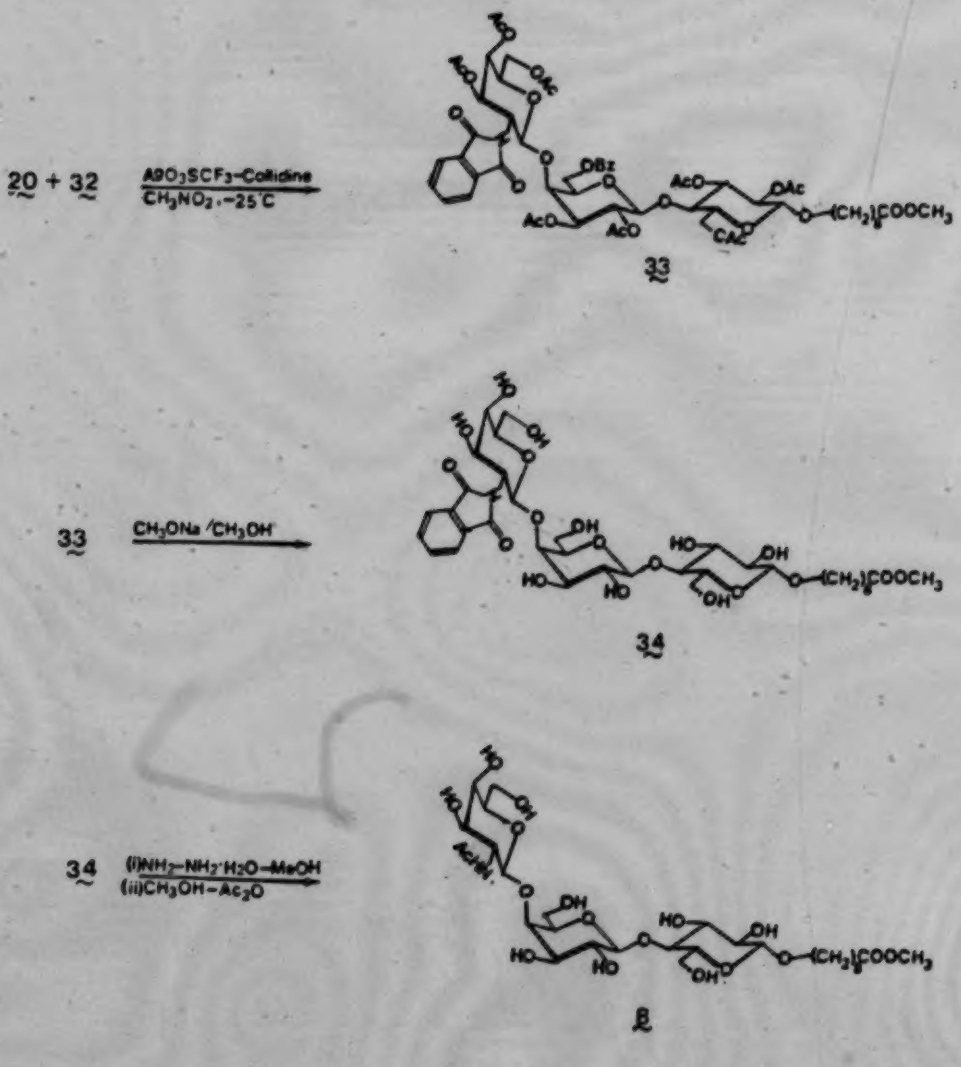
reactions, the separation of the anomeric bromides was not necessary since both the α and β bromides are expected[61] to provide stereoselectively only the β-glycoside due to the steric demand of the phthalimido group.

4. 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-galactopyranosyl(1→4)-β-D-galactopyranosyl(1→4)-β-D-glucopyranoside — Asialo-G_{M2} hapten (8)

The synthesis of the title compound 8 (see Scheme 8) depended to a large extent on success in the establishment of a β-glycosidic linkage on reaction of the glycosyl-bromide (32) and the alcohol (20) to form 33. De-O-acetylation followed by N-acetylation would then provide 8.

The condensation of the phthalimido bromide 32 with the alcohol 20 was carried out according to the procedure described by Lemieux and co-workers[61] which involved the use of silver triflate-collidine complex to promote the reaction. After 18 h, examination of the reaction mixture by tlc indicated the consumption of most of the alcohol and the formation of a single major product. Purification of the reaction product at this time by chromatography provided a major product as an amorphous material in 64% yield.

The ^{1}H-nmr spectrum of the above product, reproduced in Fig. 17, indicates the compound to be essentially pure.


20 + 32
AgO3SCF3-Collidine
CH3NO2, -25°C
33
CH3ONa/CH3OH
34
(i)NH2-NH2·H2O-MeOH
(ii)CH3OH-Ac2O
8
(CH2)8COOCH3

Scheme 8

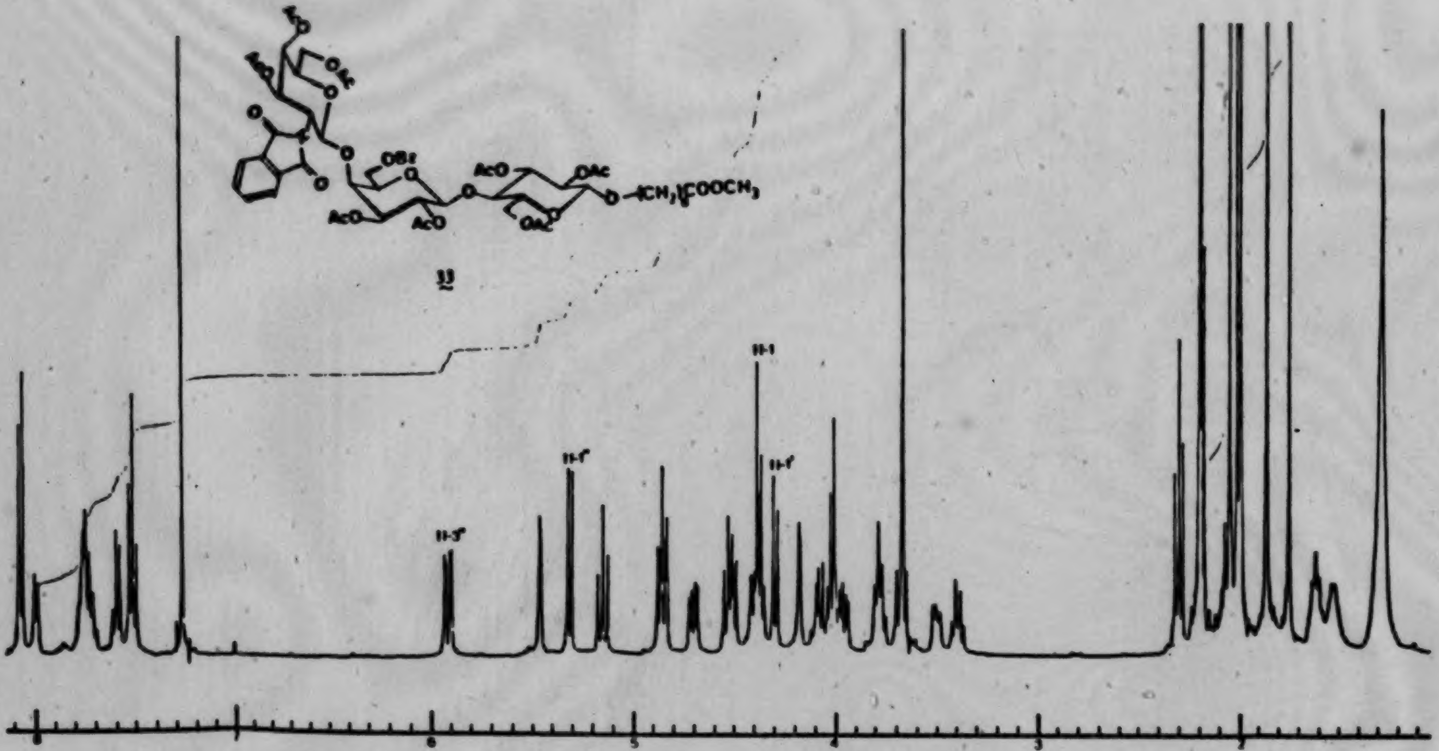


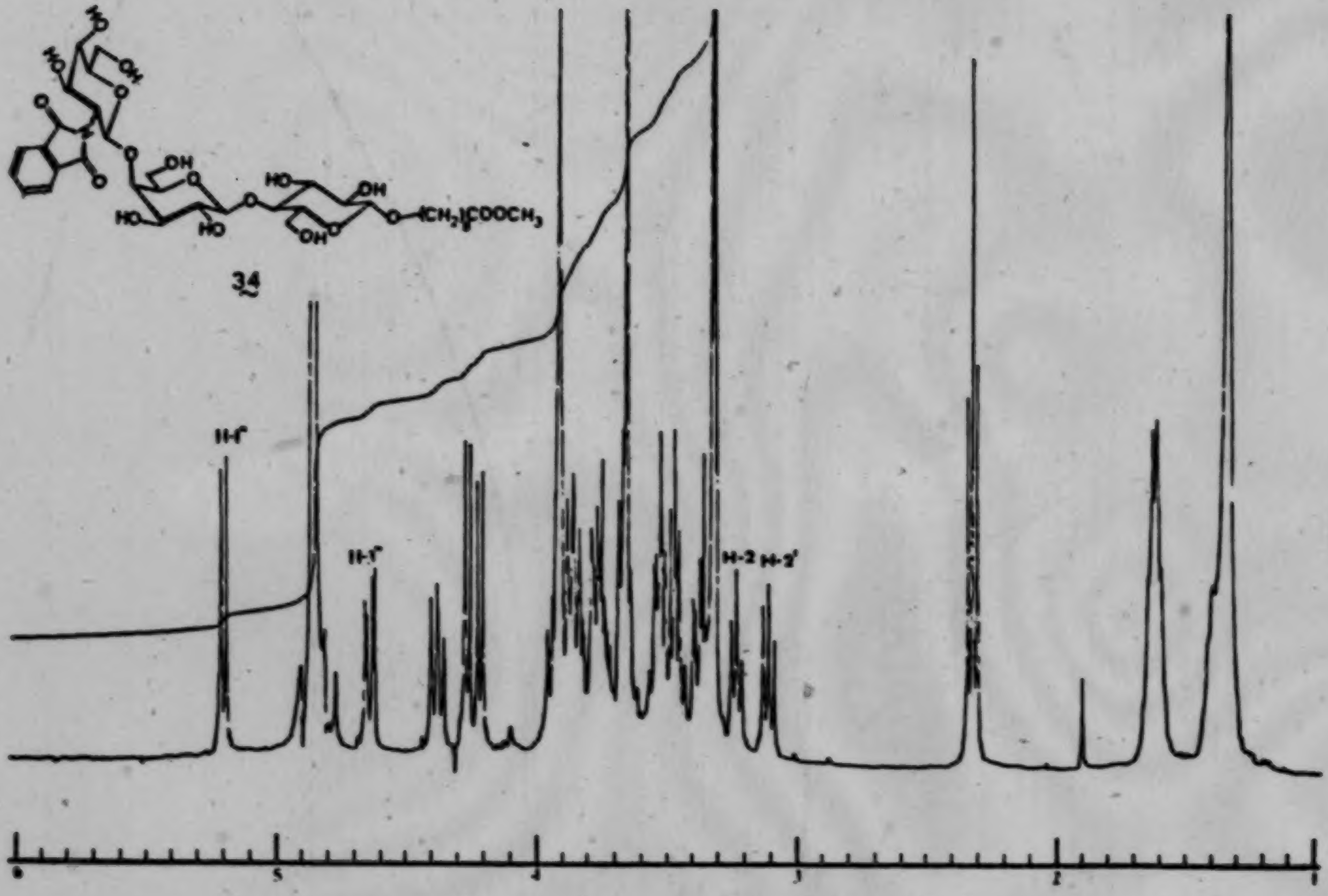
Fig.17 400 MHz ^{1}H-nmr spectrum of compound 33 in $CDCl_3$.

A comparison of the integrals of the signals between 7.0 and 8.5 ppm with that of the multiplets around 5.9 ppm requires the presence of 9 aromatic hydrogens. Also, the relative intensities of the acetyl signals around 2 ppm accounted for 21 hydrogens, as expected for the structure 33. From a comparison of this spectrum with that of allyl phthalimido galactoside (29), it can be concluded that the quartet at 5.9 ppm (spacings, 3.5 and 11.0 Hz) belongs to H-3", since this hydrogen is expected to be deshielded by an adjacent phthalimido group. The small and a large coupling constants are in accord with the presence of syn-clinal (H-4") and anti-periplanar (H-3") hydrogen atoms. The signals of the remaining hydrogens in the terminal galactosamine residue could be readily established by decoupling experiments. On this basis, the broad doublet at 5.4 ppm and the doublet at 5.32 ppm were assigned to H-4" and H-1", respectively. Consequently, the β-configuration at the newly established glycosidic center was confirmed by the coupling constant observed for the anomeric hydrogen H-1" ($J_{1'',2''}$ = 7.8 Hz) as expected for a trans-diaxial relationship between H-1" and H-2". Furthermore, in ^{13}C-nmr, the appearance of the signal for C-4' at 75.42 ppm firmly established that the glycosylation had indeed taken place at this position. Thus the ^{1}H- and

^{13}C-nmr spectra are in accordance with structure 33.

The remaining steps involved in the conversion of 33 to 8 were relatively straight forward. This involved first the removal of the O- and N-protecting groups. As expected, the de-O-acetylation and the de-O-benzoylation of 33 to provide 34 was achieved under Zemplen's conditions with sodium methoxide in anhydrous methanol in near quantitative yield. As evidenced from the ^{1}H-nmr spectrum of crude 34 (Fig. 18), the phthalimido group remained intact under these conditions. The phthalimido group was readily removed by the transamidation procedure using hydrazine hydrate according to the conditions established by Bundle and Josephson.[99] N-Acetylation of the crude amine with acetic anhydride and the purification of the product provided asialo-G$_{M2}$ hapten as a colorless solid in 80% overall yield based on 33.

The ^{1}H-nmr spectrum of 8, which is reproduced in Fig. 19, established its identity and the high level of purity. A description of the assignment of the signals in the ^{1}H- and ^{13}C-nmr spectra of 8 (Fig. 20) is presented in the latter part of this thesis while assessing the conformational preferences of this trisaccharide. The point that needs to be emphasized here is that, as expected, all three anomeric hydrogens have chemical shifts between 4.4 and



34

Fig.18 400 MHz ^{1}H-nmr spectrum of compound 34 in CD_3OD.

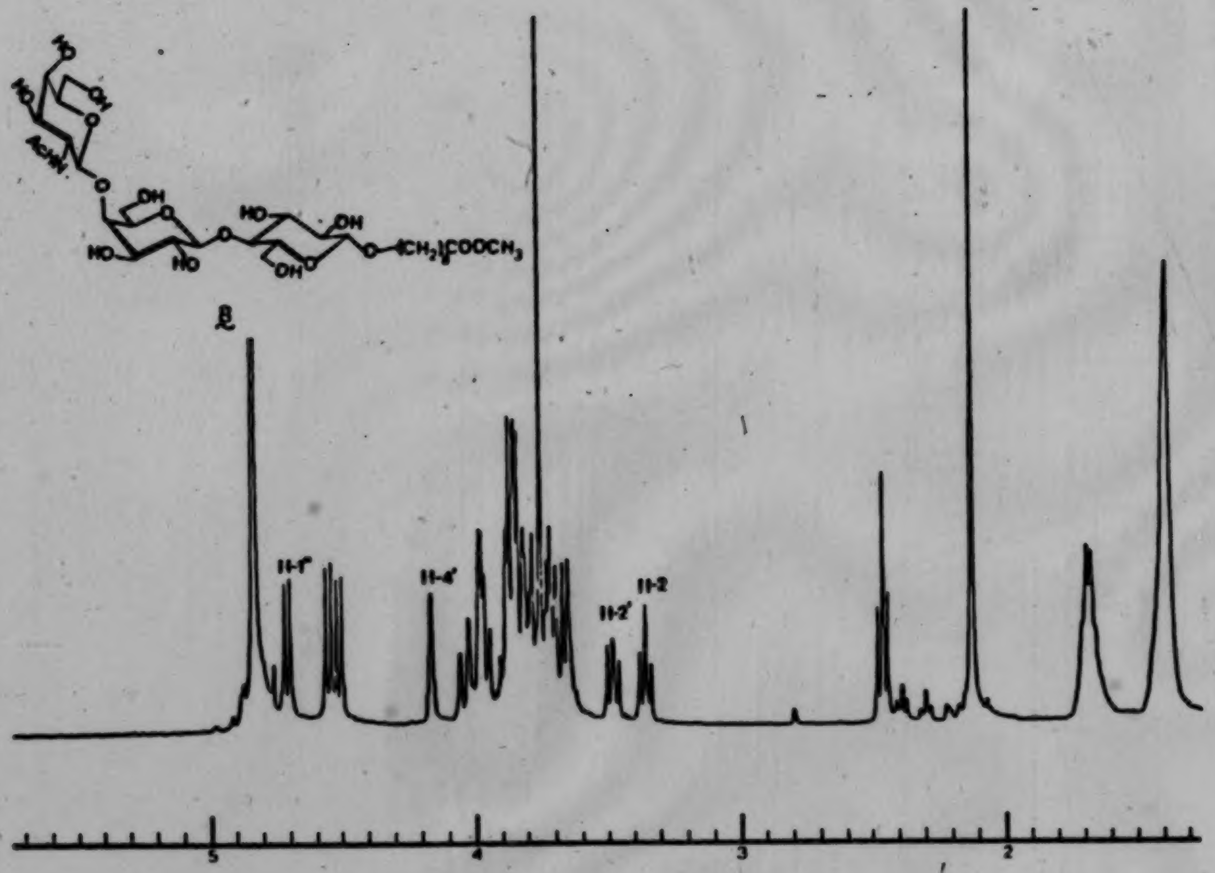
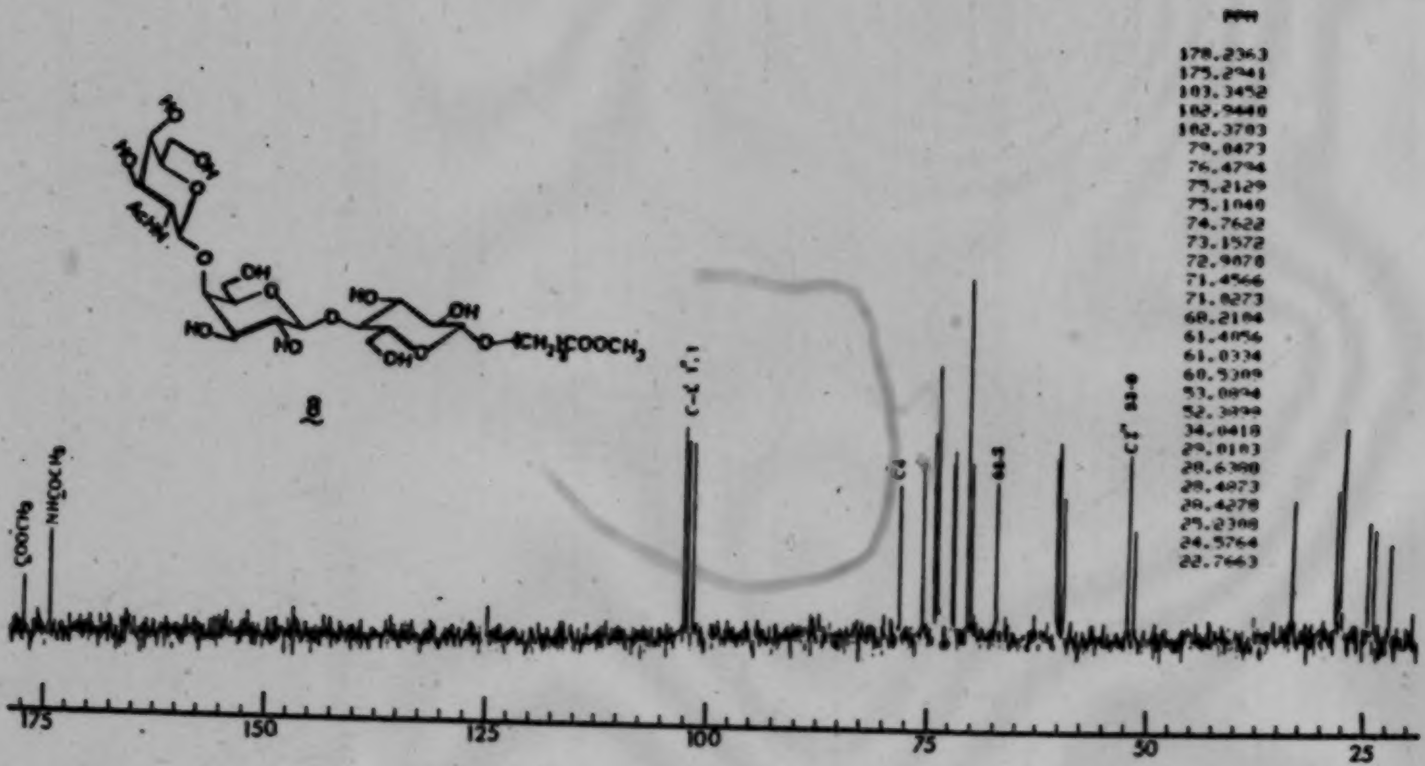


Fig. 19 400 MHz ^{1}H-nmr spectrum of asialo-G_{M2} hapten (8) in 100% deuterium oxide.

4.7 ppm and the coupling constants are all about 7.8 Hz. Thus, the β-configuration for all the glycosidic centers is firmly established. It is interesting to note that, as the result of glycosylation, the signal for H-2' of the inner galactose residue is significantly shielded by about 0.2 ppm from that observed in a normal methyl β-galactoside.[95] As will be discussed later, this shielding of H-2 of galactose is observed in all compounds having a βDGalNAc unit at the 4-position. Finally, in the ^{13}C-nmr spectrum, all three anomeric carbons appeared between 102 and 104 ppm which is typical of the carbons involved in β-glycosidic linkages.[96] The glycosylation, as expected, deshielded both of the aglyconic carbons of the sugar residues; namely, C-4 and C-4', by about 8 ppm.

The synthesis of asialo-G_{M2} did prove that the 4'-hydroxyl is fairly reactive, in contrast to what has been reported in the literature,[82] provided a proper choice of the glycosyl donor is made. It is expected that the synthetic material will be used in the near future to prepare immunochemical reagents related to G_{M2} gangliosides and, as mentioned earlier, especially in the production of antibodies directed towards the oligosaccharide portion of asialo-G_{M2} which may prove useful for the detection of certain tumor cells.



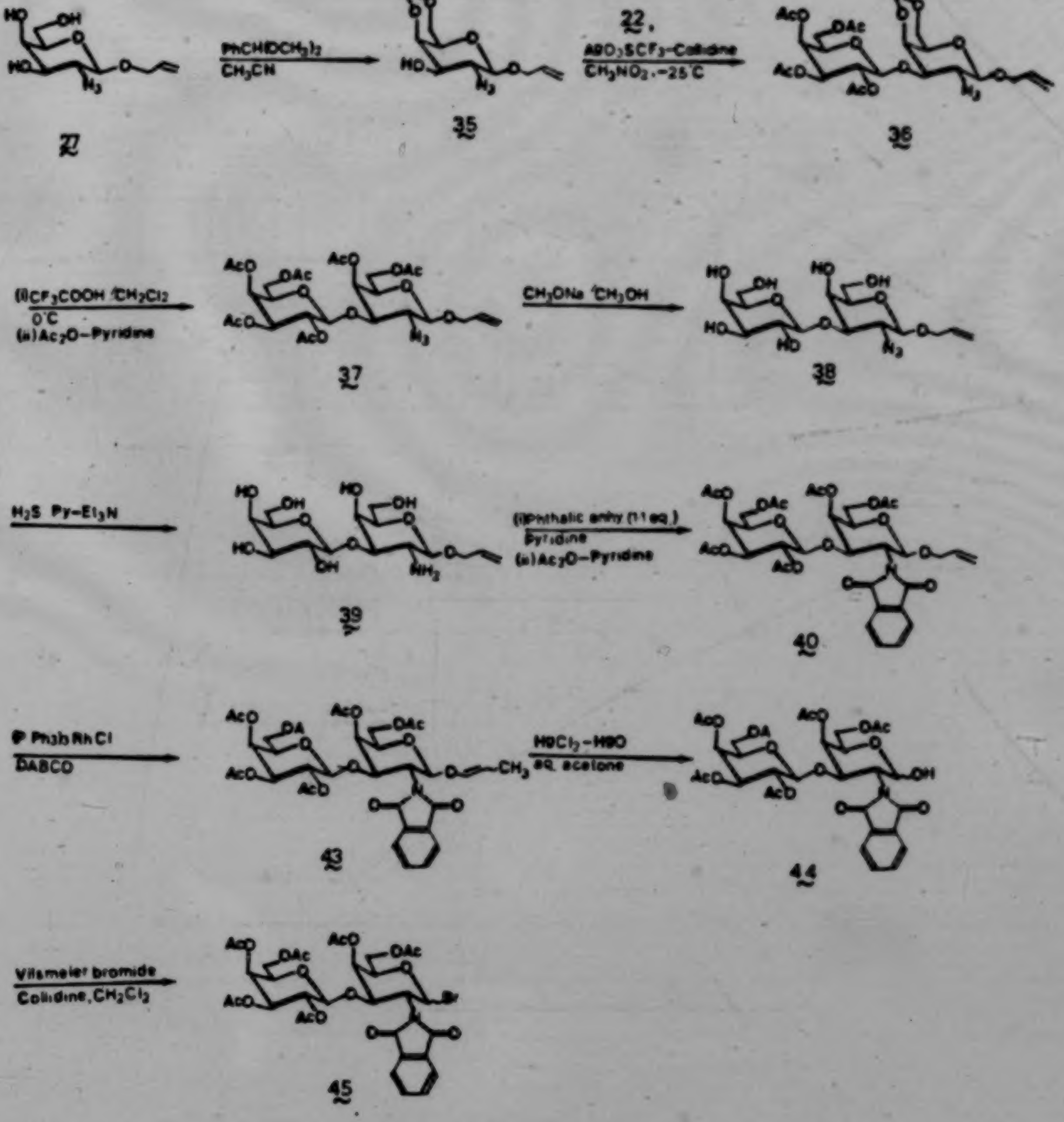
8

Fig. 20. 100 MHz ^{13}C-nmr spectrum of compound 8 in 100% deuterium oxide.

5. 4,6-Di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-phthalimido-α,β-D-galactopyranosyl bromide (**45**)

As indicated in Scheme 9, the phthalimido-bromide **45** was required for the glycosylation of the alcohol **20** to accomplish the synthesis of *asialo*-G_{M1} hapten (**9**). The allyl azidoglycoside (**27**), which was an intermediate in the synthesis of *asialo*-G_{M2} hapten (**8**), was chosen as the starting material and converted to **45** by the sequence of reactions shown.

The synthesis started with the preparation of the 4,6-*O*-benzylidene derivative of the azidoglycoside **27** to provide **35**. This was readily achieved by Evans' procedure[77] which involved a transacetalation reaction of the alcohol **27** with α,α-dimethoxytoluene in the presence of a catalytic quantity of *p*-toluenesulphonic acid. This provided a solid which crystallized from toluene in more than 79% yield. The ^{1}H-nmr spectrum of this material, reproduced in Fig. 21, indicated a high level of purity. The relative intensities of the signals require the presence of only one benzylidene ring in the molecule. The doublet at 2.59 ppm (J = 3.5 Hz) disappeared on exchange with deuterium oxide. Therefore, this signal can be assigned to the secondary hydroxyl group.


HO
OH
O
HO
N3
O
27
PhCH(OCH3)2
CH3CN
Ph
O
O
O
HO
N3
35
22,
AgO3SCF3-Collidine
CH3NO2, -25°C
AcO
OAc
AcO
AcO
N3
36
(i)CF3COOH /CH2Cl2
0°C
(ii)Ac2O-Pyridine
AcO
OAc
AcO
OAc
AcO
AcO
N3
37
CH3ONa /CH3OH
HO
OH
HO
OH
HO
HO
N3
38
H2S Py-Et3N
HO
OH
HO
OH
HO
OH
NH2
39
(i)Phthalic anhy (1.1 eq.)
Pyridine
(ii)Ac2O-Pyridine
AcO
OAc
AcO
OAc
AcO
AcO
N
O
O
40
(Ph3P)3RhCl
DABCO
AcO
OA
AcO
OAc
AcO
AcO
N
CH3
O
O
43
HgCl2-HgO
aq. acetone
AcO
OA
AcO
OAc
AcO
AcO
OH
N
O
O
44
Vilsmeier bromide
Collidine, CH2Cl2
AcO
OAc
AcO
OAc
AcO
AcO
Br
N
O
O
45

Scheme 9

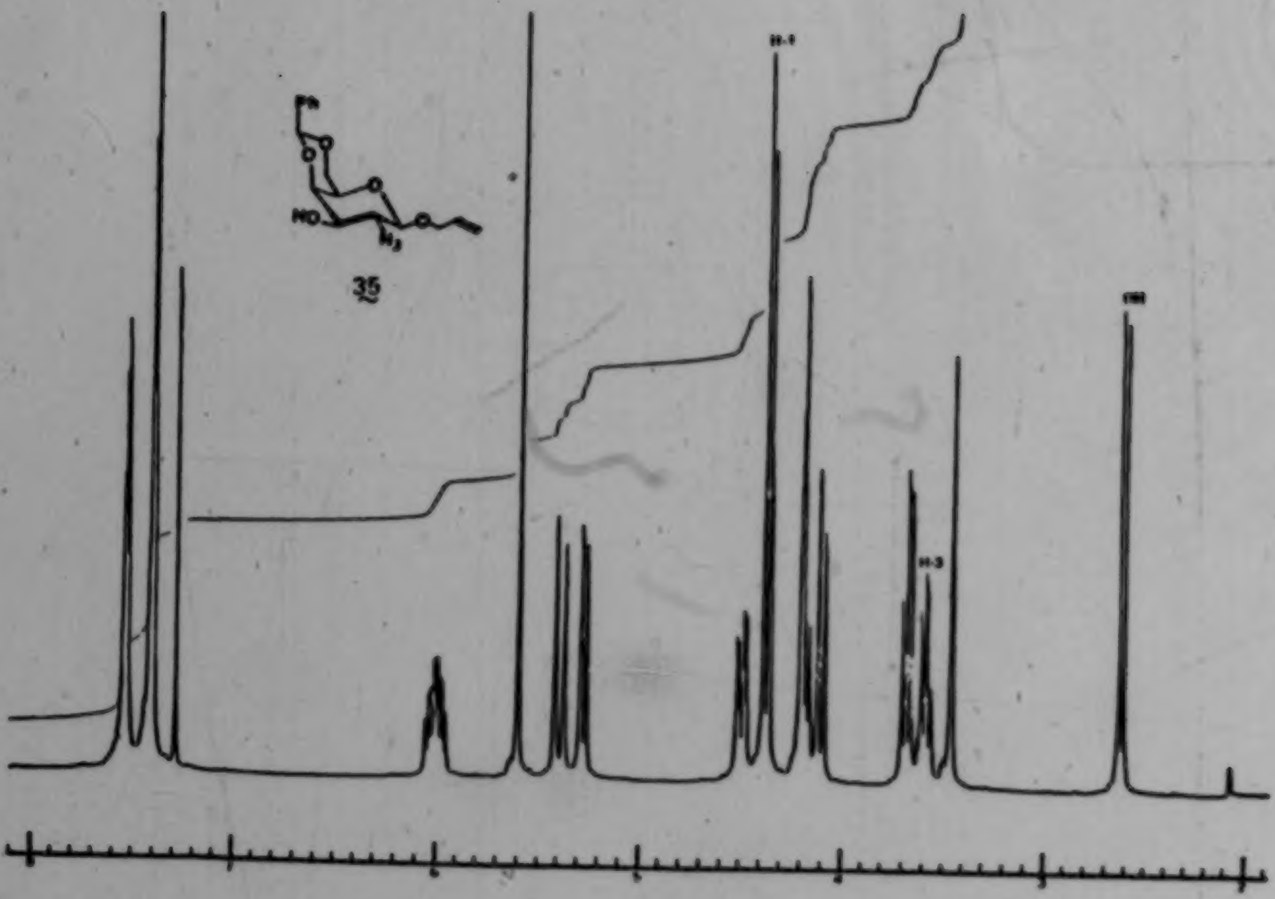


Fig.21 400 MHz ^{1}H-nmr spectrum of compound 35 in $CDCl_3$.

This hydroxyl hydrogen was coupled to the multiplet at 3.56 ppm since, after deuterium oxide exchange, it had collapsed to a quartet (dd, J = 11.0, 3.5 Hz). In view of the spacing, the hydroxyl group was at the 3-position. Therefore, the product obtained in the above transacetalation reaction was the 4,6-*O*-benzylidene derivative. This is in accordance with the observation that the hydrogens at the 4- and 6-positions are deshielded by about 0.3 - 0.6 ppm relative to their positions in the spectrum of the starting material. The structural assignment of **35** is also confirmed by its ^{13}C-nmr spectrum. As mentioned earlier, it is known that C-6 of β-D-galactopyranosides appear around 61±1 ppm. In fact, for compound **27**, the signal for C-6 is observed at 60.26 ppm whereas in compound **35**, C-6 is at 69 ppm. Also, the introduction of the benzylidene group led to deshielding of H-4 by about 7 ppm.

The next step was to glycosylate the alcohol **35** with a suitably protected α-D-galactopyranosyl bromide. Lemieux, Baker and Ratcliffe[78] have reported conditions for the glycosylation of an alcohol, similar to **35**, with acetobromogalactose to provide, in greater than 70% yield, a product related to the T antigen (βDGal(1→3)αDGalNAc-OR). The procedure, known as the Helferich modification of the Koenigs-Knorr reaction, involved the use of $Hg(CN)_2$ as promoter. However, when the condensation of **35** with the bromide **22** was attempted, a complex mixture of products was obtained and their separation proved very difficult. A major product was identified (^{1}H-nmr) as 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (**22b**). In addition, two disaccharide products (**36** and **36a**) were obtained in about 50% total yield. The ^{1}H-nmr spectra of these two compounds, reproduced in Figs. 22 and 23, indicate a high level of purity. The presence of only one benzylidene and four acetyl groups is indicated. The spectrum for compound **36** (Fig. 22) shows doublets at 4.8 ppm ($J = 7.8$ Hz) and 4.33 ppm ($J = 7.8$ Hz)(Table 2) which were assigned to H-1' and H-1 and confirmed by decoupling experiments. The coupling constants require the β-configuration for both of the anomeric centers. Also, the ^{13}C-chemical shifts of anomeric carbons (101.2 ppm and 100.8 ppm)(Table 3) support this conclusion.

The presence of only one signal in the ^{1}H-nmr spectrum of **36a** in the region typical for an anomeric hydrogen of a β-glycoside; namely, that at δ4.4 ($J = 7.8$ Hz)(Fig. 23) suggested that the newly formed glycosidic bond was in the α-configuration. This was best established to be the case by examination of the spectrum (Fig. 24) of the product (**37a**) obtained on de-*O*-acetylation. A signal typical for an anomeric hydrogen of an α-glycoside was now evident at 5.11 ppm

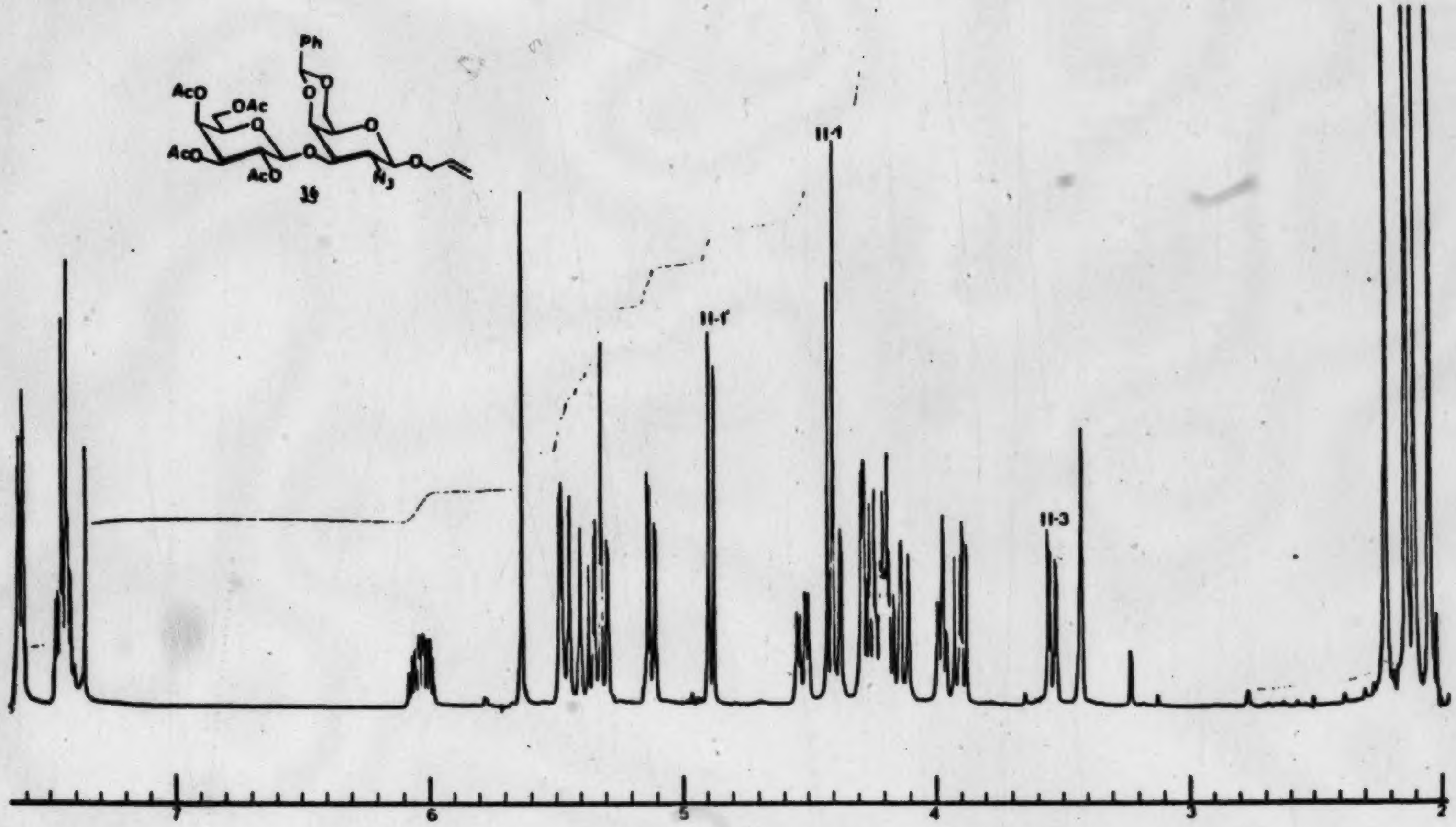


Fig.22   400 MHz ^{1}H-nmr spectrum of compound 36 in CDCl$_3$.

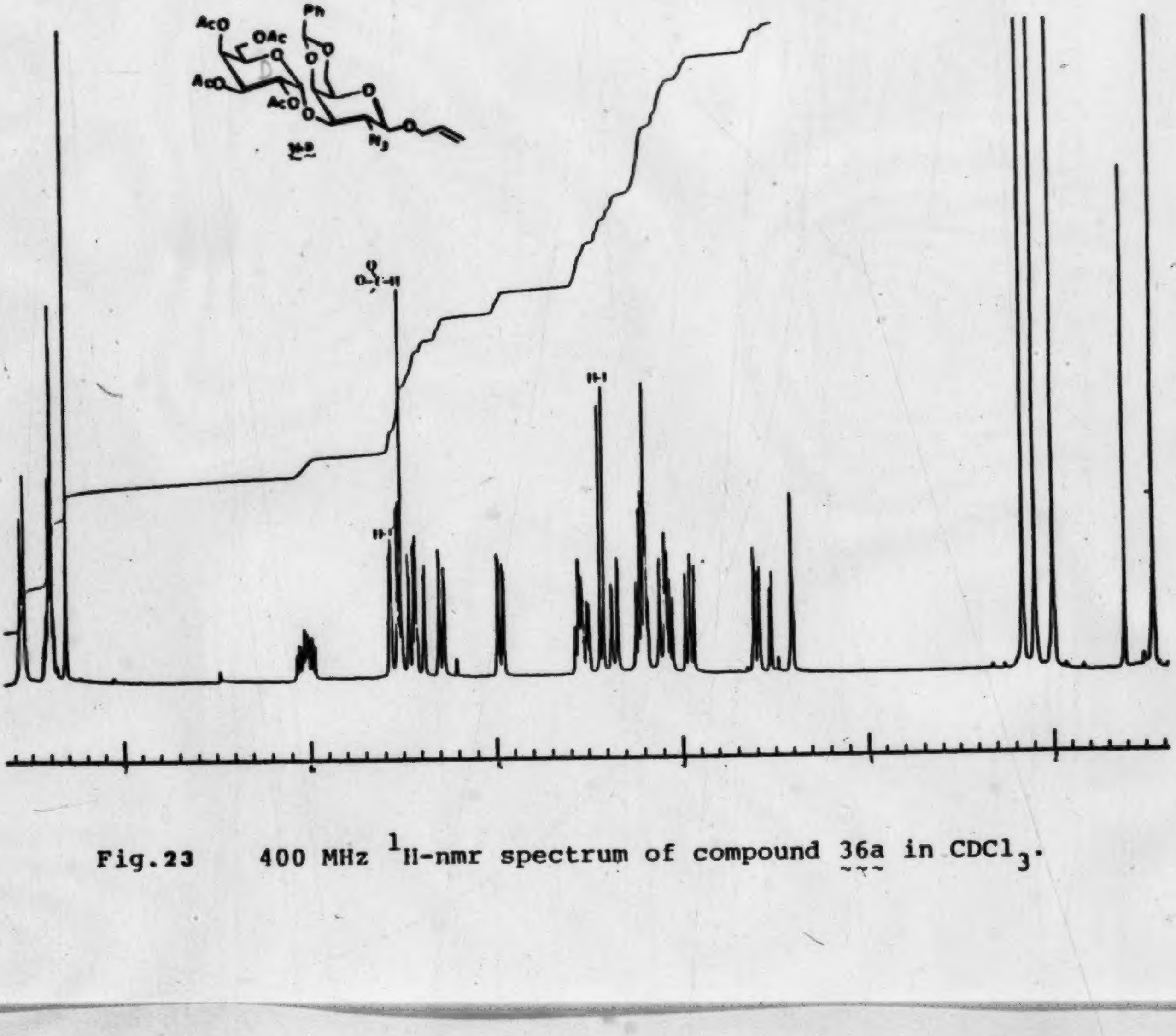


Fig.23 400 MHz ^{1}H-nmr spectrum of compound 36a in CDCl$_3$.

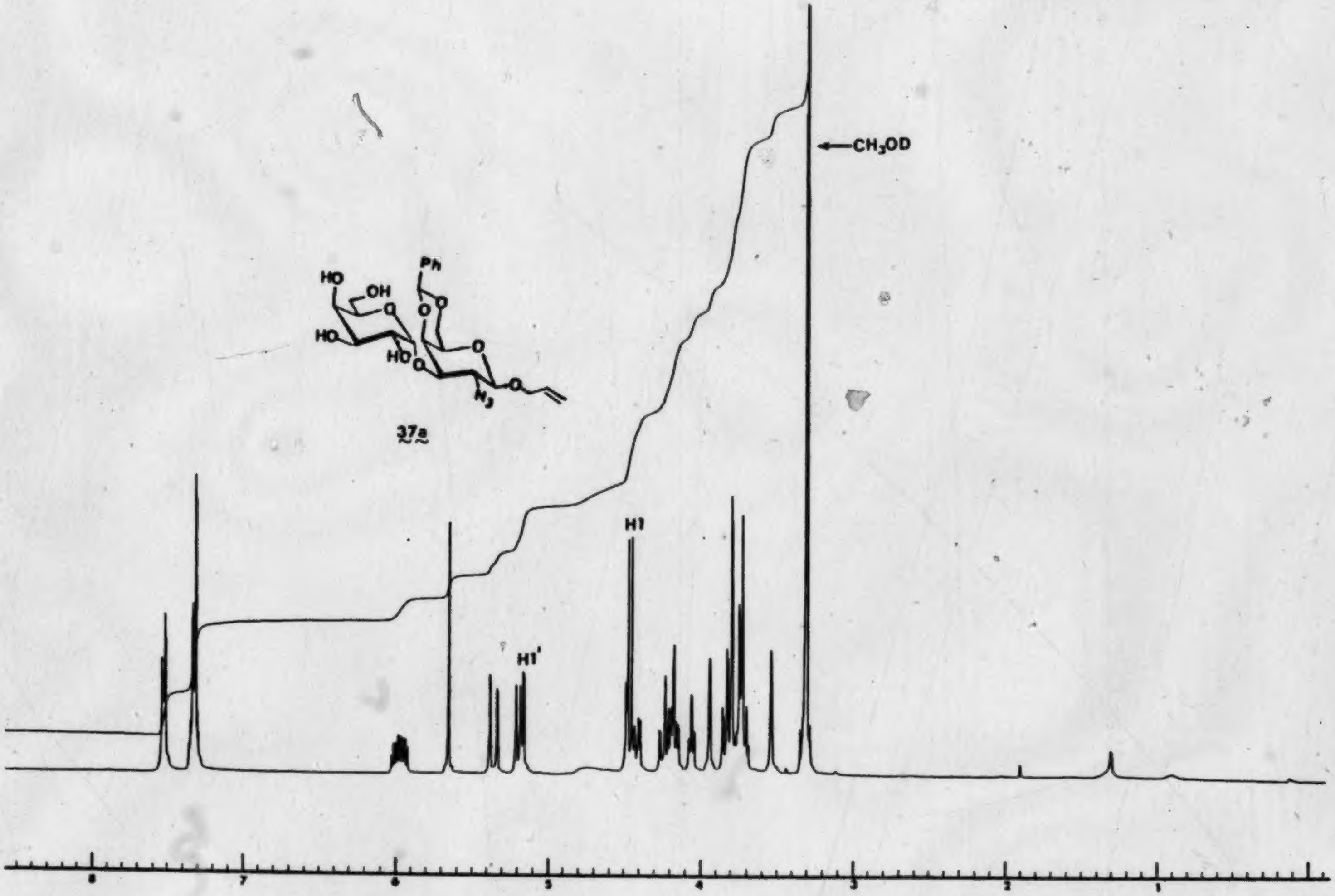


Fig. 24 360 MHz ^{1}H-nmr spectrum of compound 37a in CD_3OD. The signal for HOD is suppressed.

TABLE 2

Comparison of ^{1}H-chemical shifts of compounds 36, 36a and 36b.

Compound	H-1'	H-2'	H-3'	H-4'	H-4'	H-6'a	H-6'b	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
36	5.58	5.38	4.90	5.44	4.50	4.16	4.04	4.4	3.9	3.56	4.16	3.38	4.31	4.04
36a	4.80	5.26	5.02	5.39	3.89	4.09	4.19	4.33	3.82	3.46	4.21	3.36	4.31	4.04
36b	5.12	5.88	5.60	6.0	4.38	4.78	4.38	4.28	3.78	3.48	4.24	3.15	4.20	3.80

TABLE 3

Comparison of ^{13}C-chemical shifts of sugar carbons of compounds 36, 36a, and 36b

36	101.23 (C-1)	100.32 (C-1')	78.63 (C-3)	75.11 (C-4)	71.03 (C-5')	70.10	69.01 (C-6)	68.95 (C-3') (C-2')	67.17 (C-4')	62.44 (C-2)	61.41 (C-6')	
36a	101.48	91.73	73.71	70.42	70.44	69.19	68.36	68.21	67.49	66.82	61.74	61.15
36b	102.94	101.31	79.22 (C-3)	75.09 (C-4)	71.82 (C-5)	71.59 (C-5')	69.99	69.68	68.80	68.18	62.33 (C-2)	61.78 (C-6')

(J = 2.7 Hz). Consequently, the structure of this product as an α-glycoside could be assigned. It is well known[81] that the Helferich conditions often lead to the formation of an appreciable amount of the α-glycoside. Some of the ^{1}H-nmr parameters of **36a** are very striking (see Table 2). For example, as compared to H-5' of **36**, H-5' of **36a** is 0.61 ppm to higher field. Also, one of the acetyl signals appears at an unusually high field (1.45 ppm). These anomalous values undoubtedly require these hydrogens to be in the shielding region of the phenyl group and thereby confirm the conformation drawn in Fig. 23.

Thus, three major products (**22c**, **36** and **36a**) were formed under Helferich conditions and the yield of the desired compound (**36**) was far from satisfactory. Instead of pursuing this reaction further, attention was turned to making glycosides by another modification of the classical Koenigs-Knorr reaction.

Garegg and Norberg[100] described a procedure where they demonstrated that the condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**22a**) with a variety of sugar alcohols, promoted by silver triflate-collidine complex,[61] provided excellent yields of β-linked galactosides. These results were considered to be due to the better participating ability of a 2-benzoate group in the course of the glycosylation reactions. Indeed, when the condensation of the azido alcohol 35 with the benzoate 22a was performed, the desired product (36c) was obtained, after chromatographic purification, in greater than 70% yield as one single major product. The ^{1}H-nmr spectrum of 36c, reproduced in Fig. 24, confirms the β-configuration at both of the anomeric centers as the signals for H-1' and H-1 appear at 5.14 and 4.28 ppm, respectively, each with a coupling constant of 7.8 Hz.

In order to better evaluate the above condensation procedure, it was decided to perform the glycosylation of 35 with acetobromogalactose and compare the yield with that obtained with benzobromide 22a. In fact, 22 also proved to be as excellent a glycosyl donor as the benzobromide 22a and provided the desired glycoside 36 in greater than 70% yield. The structure of this product could be readily ascertained by a comparison of its ^{1}H-nmr spectrum with that of the sample obtained by way of the Helferich reaction. Thus, in our hands, both acetobromogalactose (22) and benzobromogalactose (22a) proved suitable for the glycosylation of 35. Since 22 could be prepared[101] more easily from the commercially available D-galactose, it was used to prepare large amounts of 36.

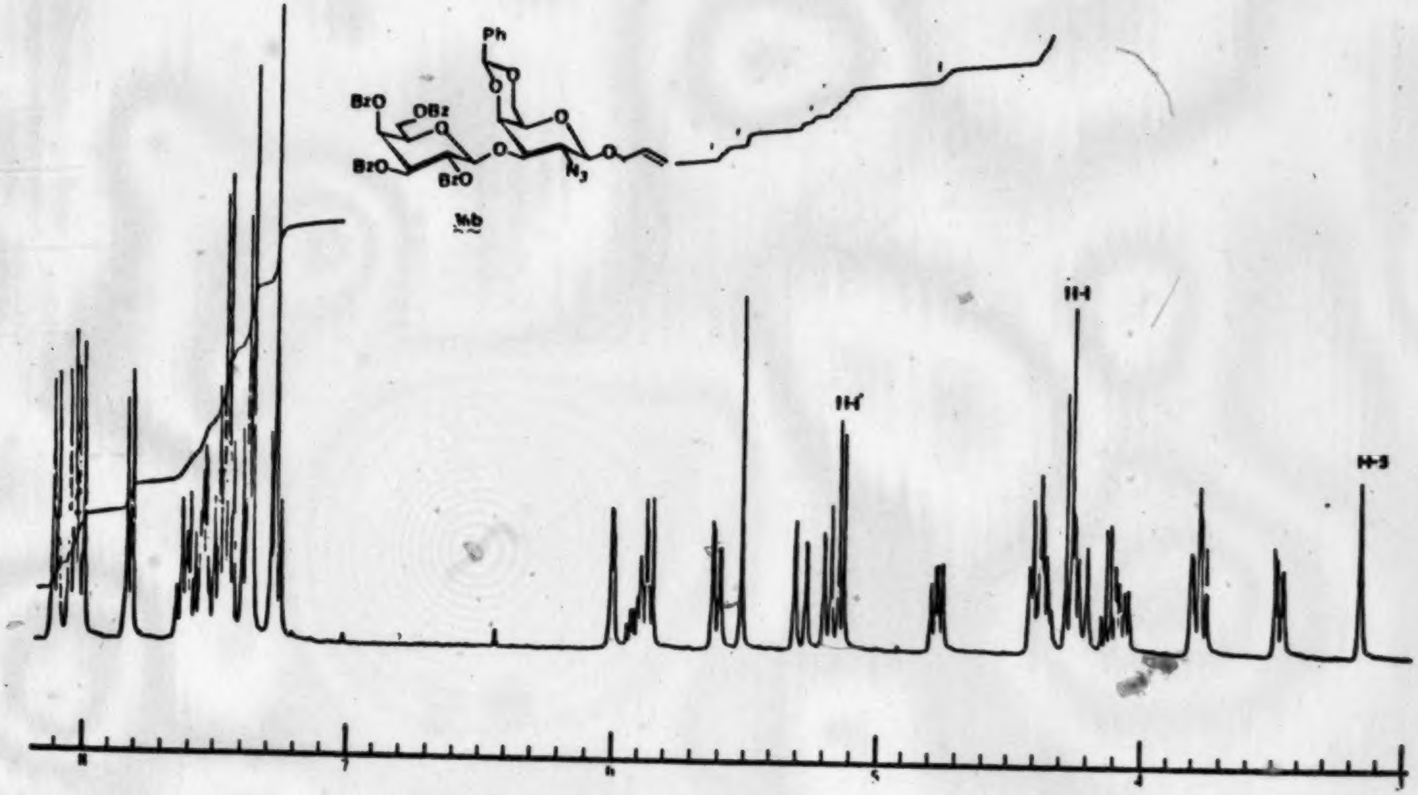


Fig. 25 400 MHz ^{1}H-nmr spectrum of compound 36b in $CDCl_3$.

As mentioned earlier, it was necessary to remove the ester protecting groups of **36** before the reduction of the azide to the amine since the intermediate amino acetate might undergo *O*- to *N*-acyl migration with the formation of a stable acetamido group. Two sets of experiments were then performed; one in which the 4,6-*O*-benzylidene group was removed prior to reduction of the azide to amine group, and the other in which removal of the benzylidene group was accomplished after the formation of the phthalimido group.

As presented in Scheme 9, the 4,6-*O*-benzylidene group of **36** was removed with aqueous trifluoroacetic acid[75] and the resulting product, on acetylation, provided **37** as a colorless crystalline solid in 80% overall yield. The structural identity and purity was established from its ^{1}H-nmr spectrum (Fig. 25). De-*O*-acetylation of **37** with sodium methoxide in methanol then provided **38** in near quantitative yield. The ^{1}H-nmr spectrum (Fig. 26), as expected, shows signals for the anomeric hydrogens at 4.4 and 4.6 ppm (J = 7.8 Hz). The two weakly coupled signals expected for the hydrogen at the 4- and 4'-positions are readily identified at 3.85 and 4.10 ppm, respectively, by comparison of these shifts with those for the H-4 atoms of β-D-galactopyranoside and methyl 3-*O*-methyl-β-D-galactopyranoside.[102] It is

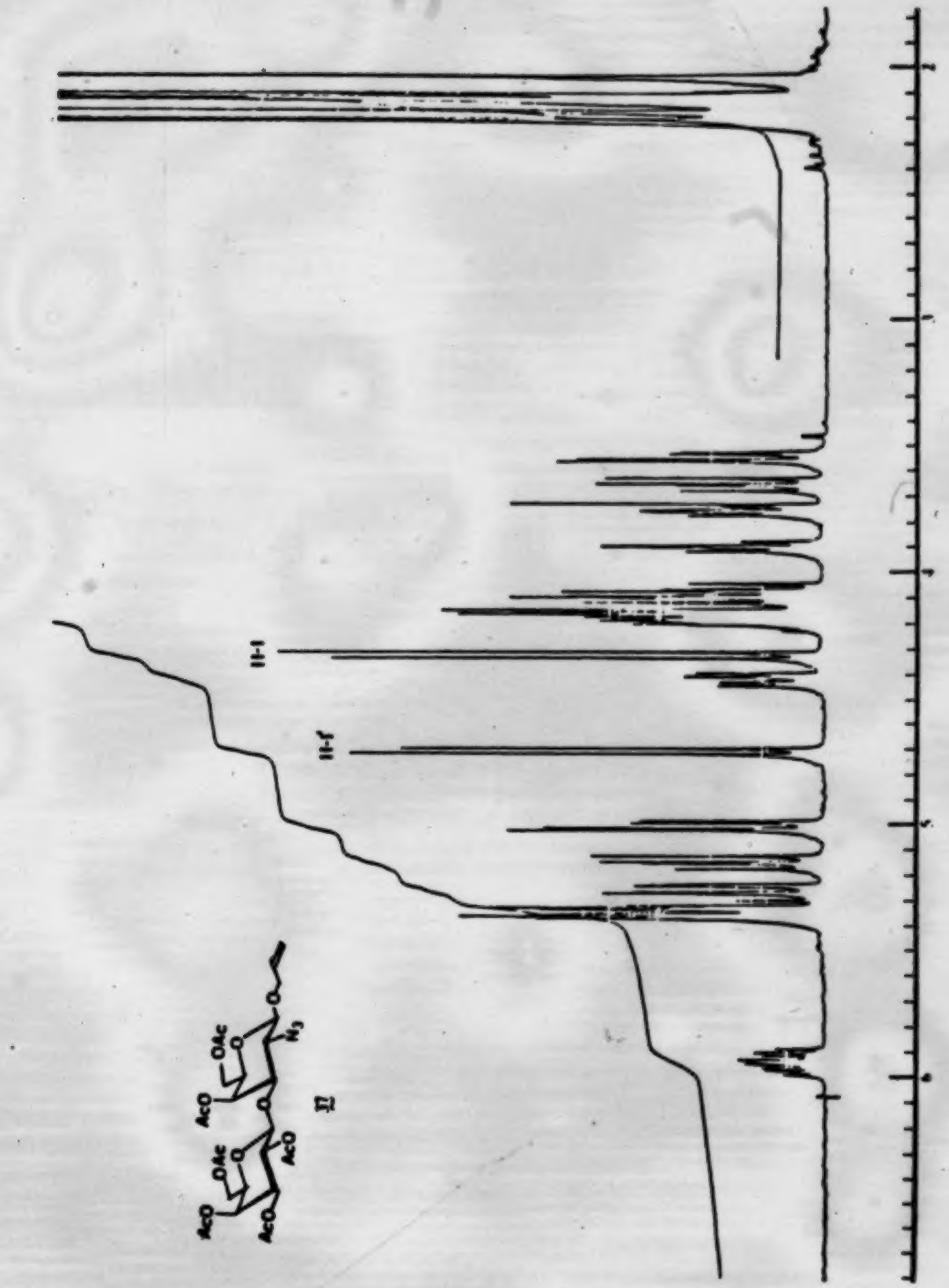


Fig. 26 360 MHz ^{1}H-nmr spectrum of compound 37 in CDCl$_3$.

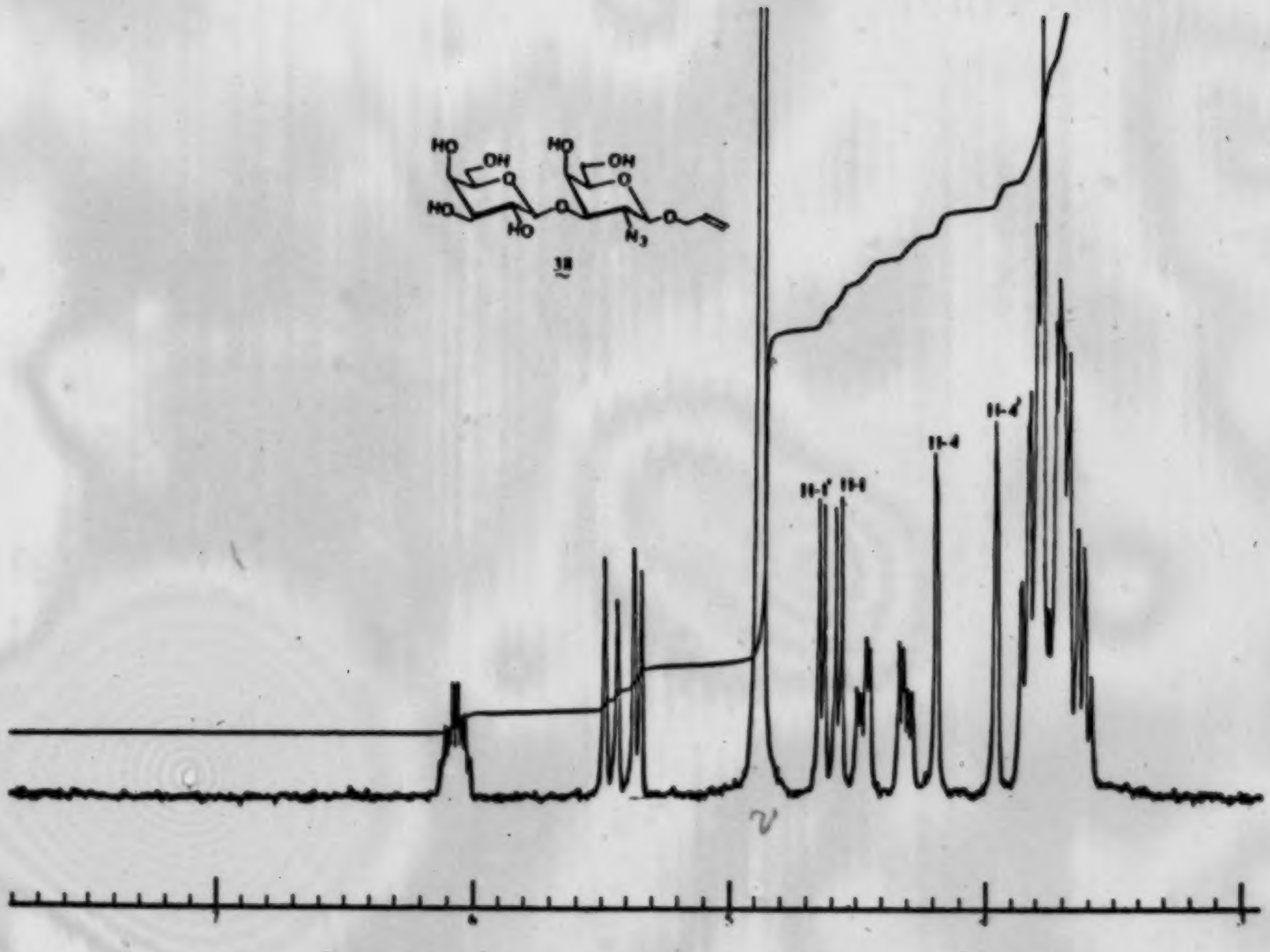


Fig. 27 360 MHz ^{1}H-nmr spectrum of compound 38 in 100% deuterium oxide.

interesting to note that this corresponds to a deshielding of H-4 compared to H-4' by 0.25 ppm and this difference was maintained in all compounds having βDGal(1→3)βDGal-OR structure in which the groups present on 4- and 4'-oxygen atoms were the same.

The reduction of the azide (38) with hydrogen sulphide[70] provided the desired amine (39). The structure was confirmed by its ^{1}H- (Fig. 27) and ^{13}C-nmr spectra. The crude amine was treated with 1.1 equivalents of phthalic anhydride to form the N-2-carboxybenzamido derivative. Subsequent treatment with acetic anhydride provided acetylated allyl phthalimidoglycoside (40) in 62% overall yield after chromatographic purification. The material crystallized from ethanol.

The presence of a phthalimido group in 40 was evident from its ^{1}H-nmr spectrum (Fig. 27). A comparison of this spectrum with that of 37 indicates significant changes in the chemical shifts of all protons which are in the proximity of the phthalimido group. Of these, the most notable are the signals for H-3, H-1 and H-2 which are deshielded by about 1.2, 0.9 and 0.9 ppm, respectively, and these observations are in accordance with expectations.[61] In contrast, the anomeric hydrogen H-1' and one of the acetyl signals are shielded by about 0.3 ppm as compared to corresponding

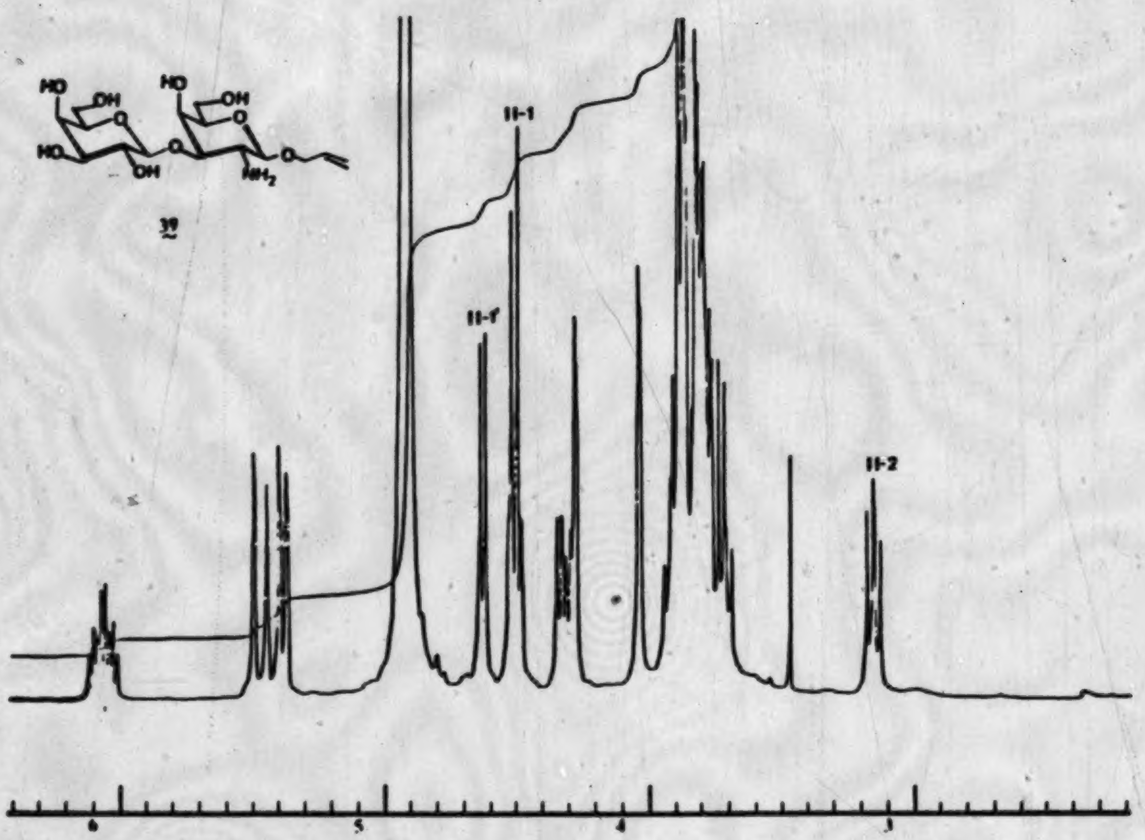


Fig. 28 360 MHz ^{1}H-nmr spectrum of compound 39 in 100% deuterium oxide.

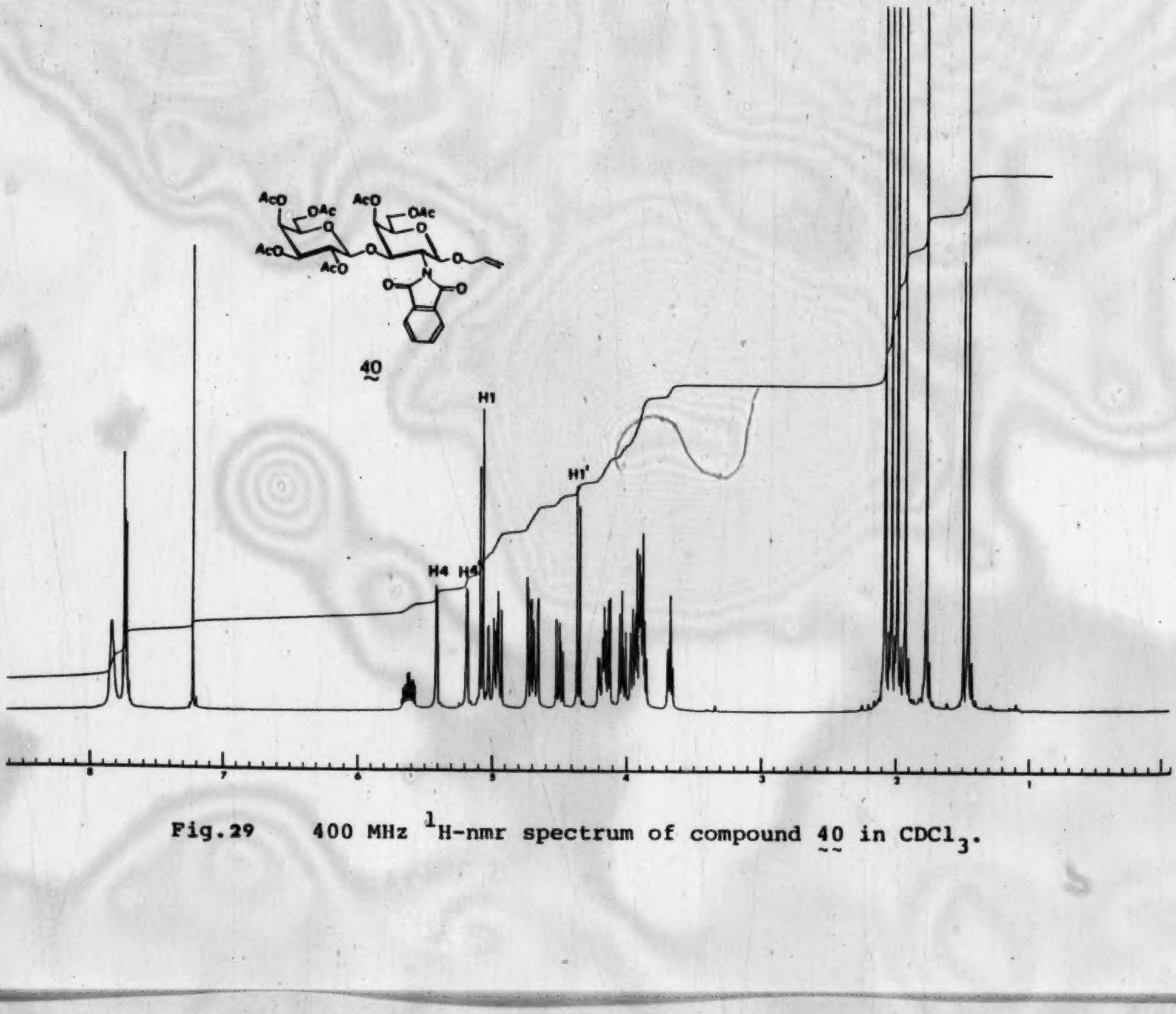
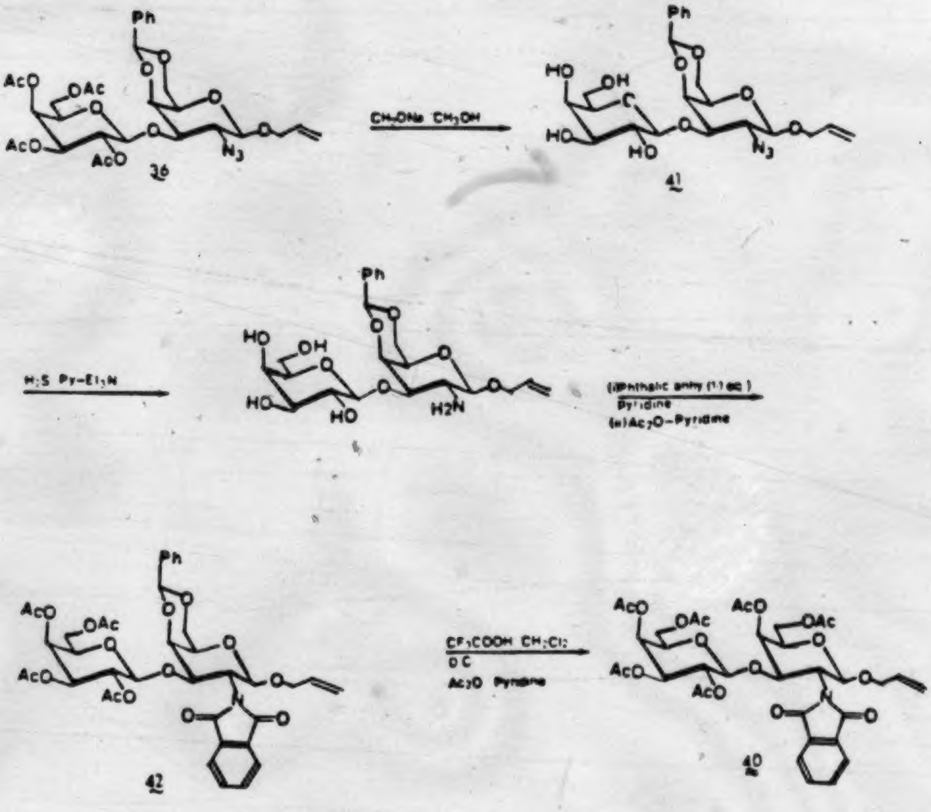


Fig.29 400 MHz ^{1}H-nmr spectrum of compound 40 in $CDCl_3$.

chemical shifts for 37. In general, the ^{1}H- and ^{13}C-nmr parameters of 40 are in good agreement with those expected.

As stated earlier, an attempt was made to prepare 40 from 36 via the benzylidene glycoside 41 as is outlined in Scheme 10.


Ph
AcO
OAc
AcO
AcO
N3
36
CH3ONa CH3OH
HO
OH
HO
HO
41
H2S Py-Et3N
H2N
(i)Phthalic anhy (1.1 eq.)
Pyridine
(ii)Ac2O-Pyridine
42
CF3COOH CH2Cl2
0 C
Ac2O Pyridine
40

Scheme 10

The de-*O*-acetylation of **36** to form **41**, the reduction of the azide to the amine, and the conversion of amine to **42** was accomplished as described earlier. But this procedure provided pure **42** (the ^{1}H-nmr spectrum is reproduced in Fig. 28) in only 45% overall yield. Treatment of **42** with aqueous trifluoroacetic acid and the acetylation of the crude product provided, after chromatographic purification, the desired hexaacetate **40** in 80% yield. Thus, the latter procedure provided **40** in slightly more than half the yield of that obtained by the procedure outlined in Scheme 9. Consequently, the former procedure involving the intermediates **37** and **38** was used for large-scale preparation of **40**.

Returning to Scheme 9, deallylation was accomplished using the procedure described for the preparation of phthalimidogalactopyranose compound **31** from **29**. The isomerization of the allyl group of **40** to 1-propenyl to form **43** was effected with *tris*-triphenylphosphine rhodium(III)chloride in the presence of 1,4-diazabicyclooctane under refluxing conditions.[69] The ratio of **40** to **43**, after 24 h, was 84:15 as was estimated from the relative intensities of signals for the H-1 atoms of **43** and **40** present in the reaction mixture (Fig. 29). This ratio did not change with longer reaction time and therefore the mixture was treated with aqueous mercuric chloride solution to selectively hydrolyze the

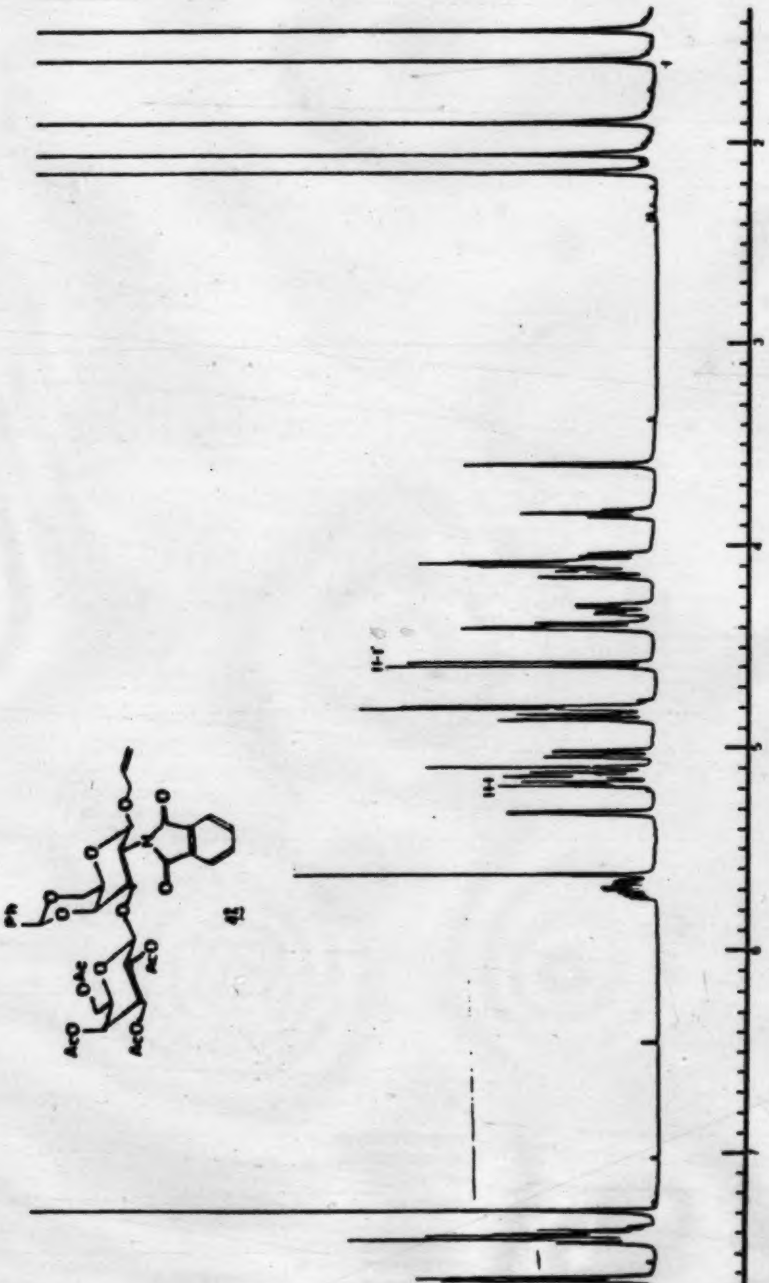


Fig. 30 400 MHz ^{1}H-nmr spectrum of compound 42 in CDCl$_3$.

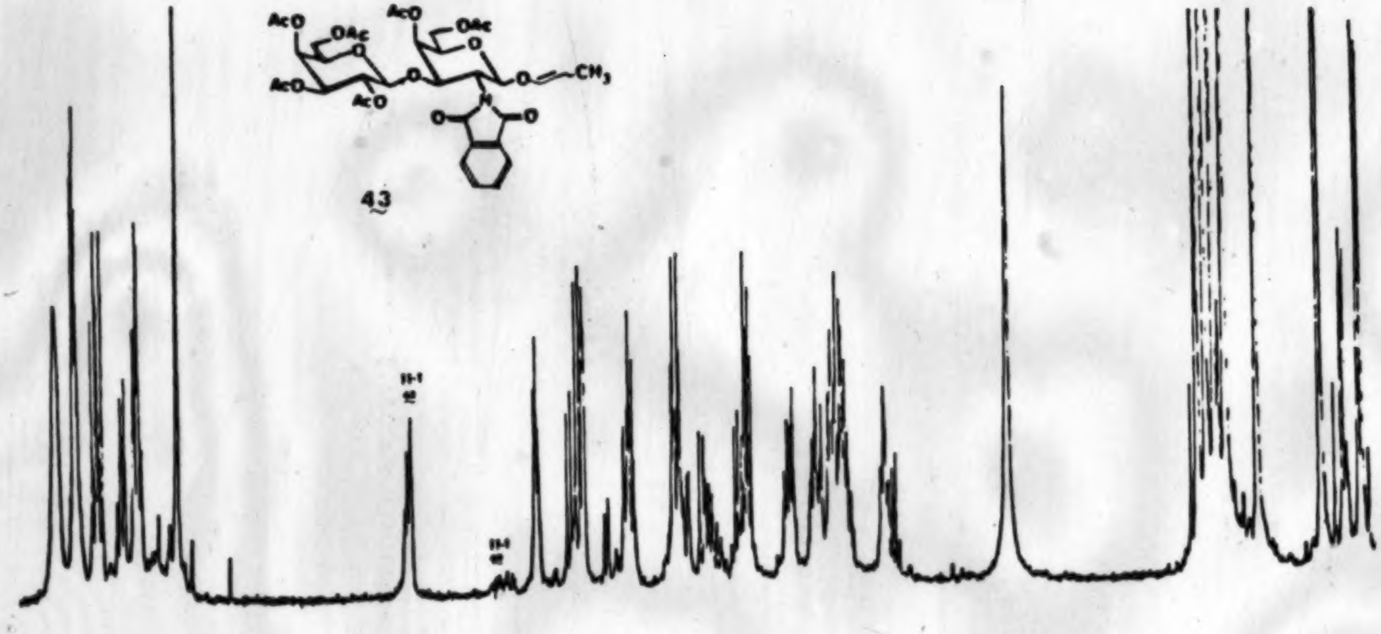
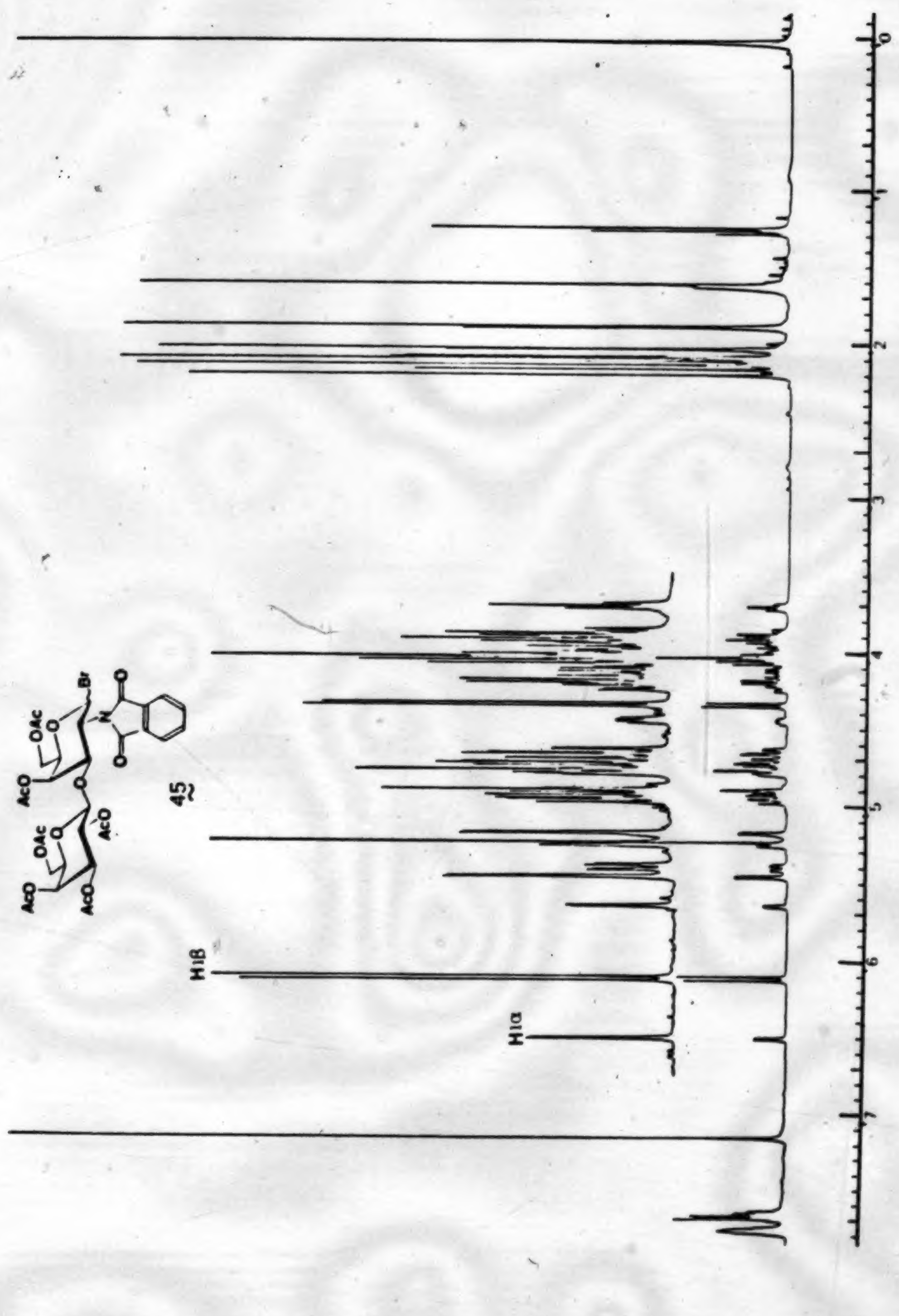


Fig.31 400 MHz ^{1}H-nmr spectrum of the reaction mixture containing compound 43 as major product, the unreacted starting material 40, tris-triphenylphosphine rhodium(III)chloride, and 1,4-diazabicyclooctane, in $CDCl_3$.

propenyl ether (43). This procedure provided, after chromatographic separation, the desired 1-hydroxy compound 44 in 84% yield. Without further characterization, when required, compound 44 was converted directly to the bromide 45 as described below.

Treatment of 44 with 1.1 equivalents of Vilsmeier bromide provided the desired bromide 45 in greater than 80% yield. The ^{1}H-nmr spectrum (Fig. 30) of the product indicated the bromide 45 to be a fairly pure mixture of the α and β anomers. The α:β anomeric ratio was estimated to be about 1:2 from the relative intensities of signals at 6.5 ppm ($J = 3.5$ Hz) and 6.1 pm ($J = 9.5$ Hz). As pointed out by Lemieux and co-workers,[61] it was not necessary to separate the anomeric bromides as both were expected to provide only the β-glycoside on condensation with the alcohol 20. Consequently, the crude mixture of bromides 45 was employed.



45

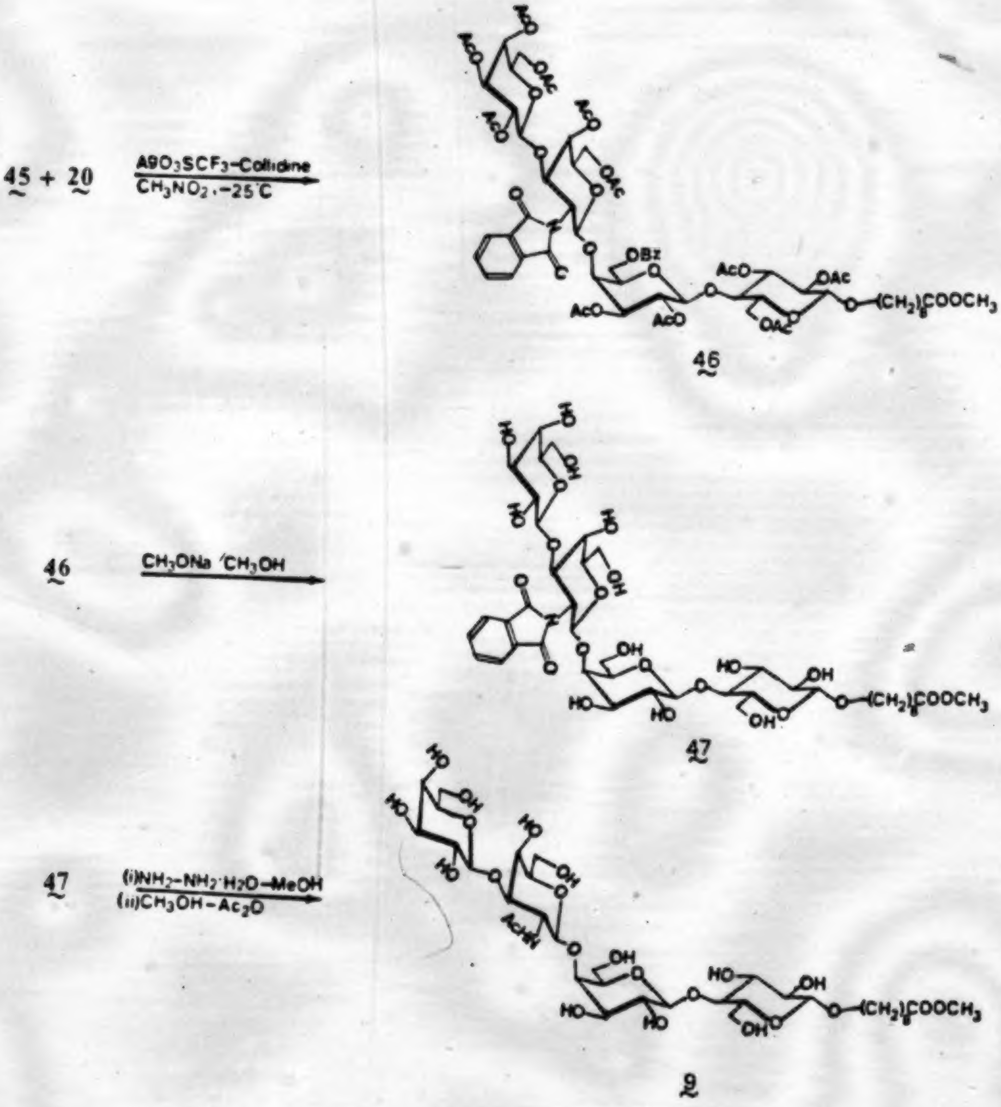
Fig.32 400 MHz ^{1}H-nmr spectrum of anomeric mixture of compound 45 in $CDCl_3$.

6. 8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl(1→4)-β-D-glucopyranoside. *Asialo*-G$_{M1}$ hapten (**9**)

After phthalimido bromide **45** (Scheme 9) and the alcohol **20** (Scheme 5) were prepared in adequate amounts, the remaining task was to synthesize the *asialo*-G$_{M1}$ hapten (**9**) as outlined in Scheme 11.

The condensation of **45** with **20** was carried out with silver triflate-collidine complex according to the procedure described by Lemieux and co-workers.[61] After 24 h, examination of the reaction mixture by tlc indicated the consumption of most of **20** and the formation of a single major product. Chromatographic purification then afforded a colorless amorphous substance in 64% yield.

The ^{1}H-nmr spectrum (Fig. 33) of the product required a high level of purity. As expected for structure **46**, integration showed the presence of 9 hydrogen atoms in the aromatic region (7.4 to 8.1 ppm) and 11 acetyl signals (33 hydrogen atoms). The signals for the 28 sugar hydrogens appeared between 3.3 and 5.5 ppm, and the assignment of most of them could not have been achieved without the availability of the high field spectrometers. Indeed, the resolution and dispersion of the hydrogen signals were sufficient at 360 MHz


45 + 20
AgO3SCF3-Collidine
CH3NO2, -25°C
46
46
CH3ONa/CH3OH
47
47
(i) NH2-NH2·H2O-MeOH
(ii) CH3OH-Ac2O
9
AcO
OAc
OBz
O-(CH2)8COOCH3
HO
OH
AcHN

Scheme 11

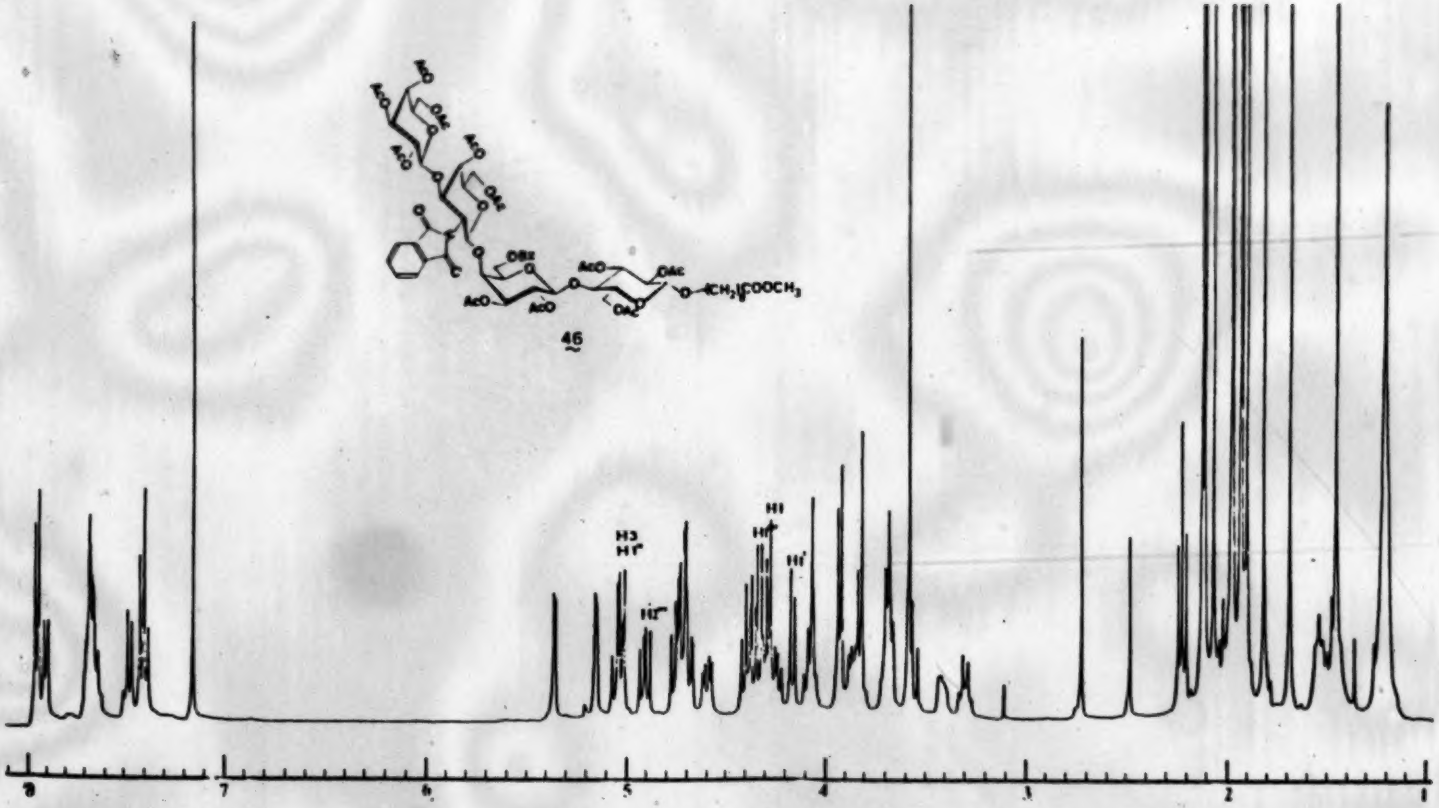


Fig. 33 360 MHz ^{1}H-nmr spectrum of compound 46 in $CDCl_3$.

to allow selective decoupling of signals to confirm the structure of 46.

A comparison of the ^{1}H-nmr spectrum of 46 (Fig. 33) with those of the allyl glycoside 40 (Fig. 29) and the alcohol 20 (Fig. 8) shows that the signals at 5.44, 5.24 and 5.12 ppm can be assigned to H-4", H-4"', and H-3 and H-1", respectively. The coupling constant (8.0 Hz) of the signal for H-1" requires an anti-periplanar relationship between this atom and H-2" and confirmed the β-configuration for the newly established glycosidic center. The chemical shifts of the remaining hydrogens could be assigned from the decoupling experiments and the estimated chemical shift values are all well in accordance with those expected for structure 46. Furthermore, the ^{13}C-nmr chemical shifts, especially for the anomeric carbons, were in excellent agreement with those expected for a typical β-galacto- and glucopyranoside and supported the above structural assignment. However, since the structure of 9, derived from 46, was firmly established, the presentation of these data in detail is considered superfluous.

Removal of the acetyl and benzoyl groups of 46 under Zemplen conditions afforded 47 in near quantitative yield. As usual, the phthalimido group was then removed using hydrazine.[99] The crude amine was not characterized.

Treatment with acetic anhydride in methanol followed by purification on a P-2 Bio-Gel column afforded a colorless solid in 85% overall yield (based on 46) which was characterized as the desired *asialo*-G_{M1} hapten (9).

The ^{1}H-nmr spectrum of 9 is reproduced in Fig. 34 and some of the parameters are presented in Table 4 along with those of structurally related compounds. It is seen that doublet signals for all four anomeric hydrogens are present between 4.3 and 4.7 ppm. The coupling constants of these signals (7.8 Hz) confirm the presence of β-glycosidic linkages. Comparison of this spectrum with that for *asialo*-G_{M2} hapten (8) shows the expected similarity except for the signals arising from the terminal galactose unit of 9. The weakly coupled equatorial H-4's of the three galactose units were identified at 4.16 (H-4'), 4.10 (H-4"), and 3.85 ppm (H-4"'). The two high field signals at 3.44 and 3.22 ppm were assigned to H-2' and H-2 by a comparison of their chemical shifts with those for compound 8. The spectra for the remaining hydrogens were too complex to be analyzed. The ^{13}C-chemical shifts for 9 (see Fig. 35) are in accordance with the structural assignment. This will be explained in detail during the discussion of the conformational analysis of 9 in the next part of the thesis.

It is hoped that the synthetic compound will prove useful for the preparation of immunochemical reagents related to gangliosides.

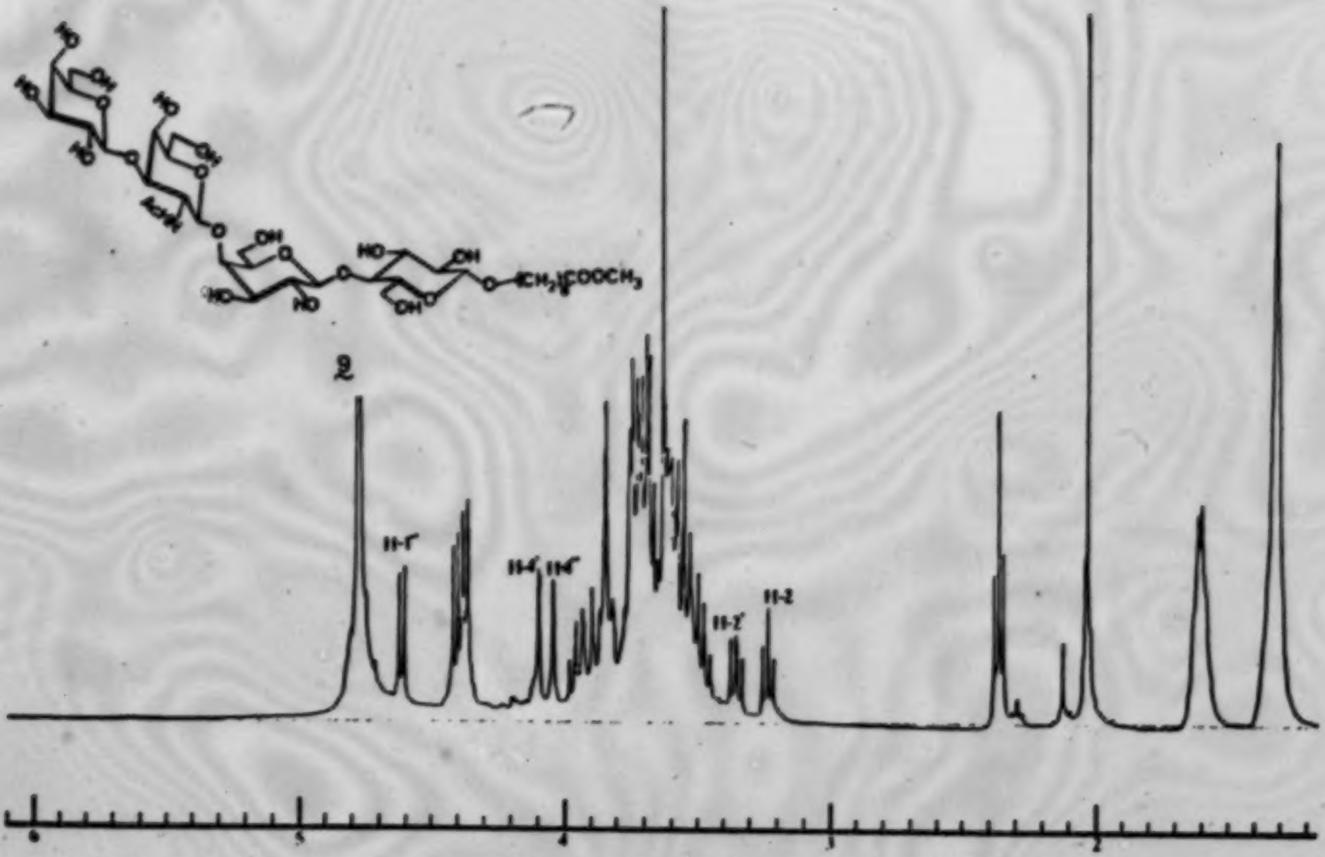


Fig.34 400 MHz ^{1}H-nmr spectrum of asialo-G_{M1} hapten (9) in 100% deuterium oxide.

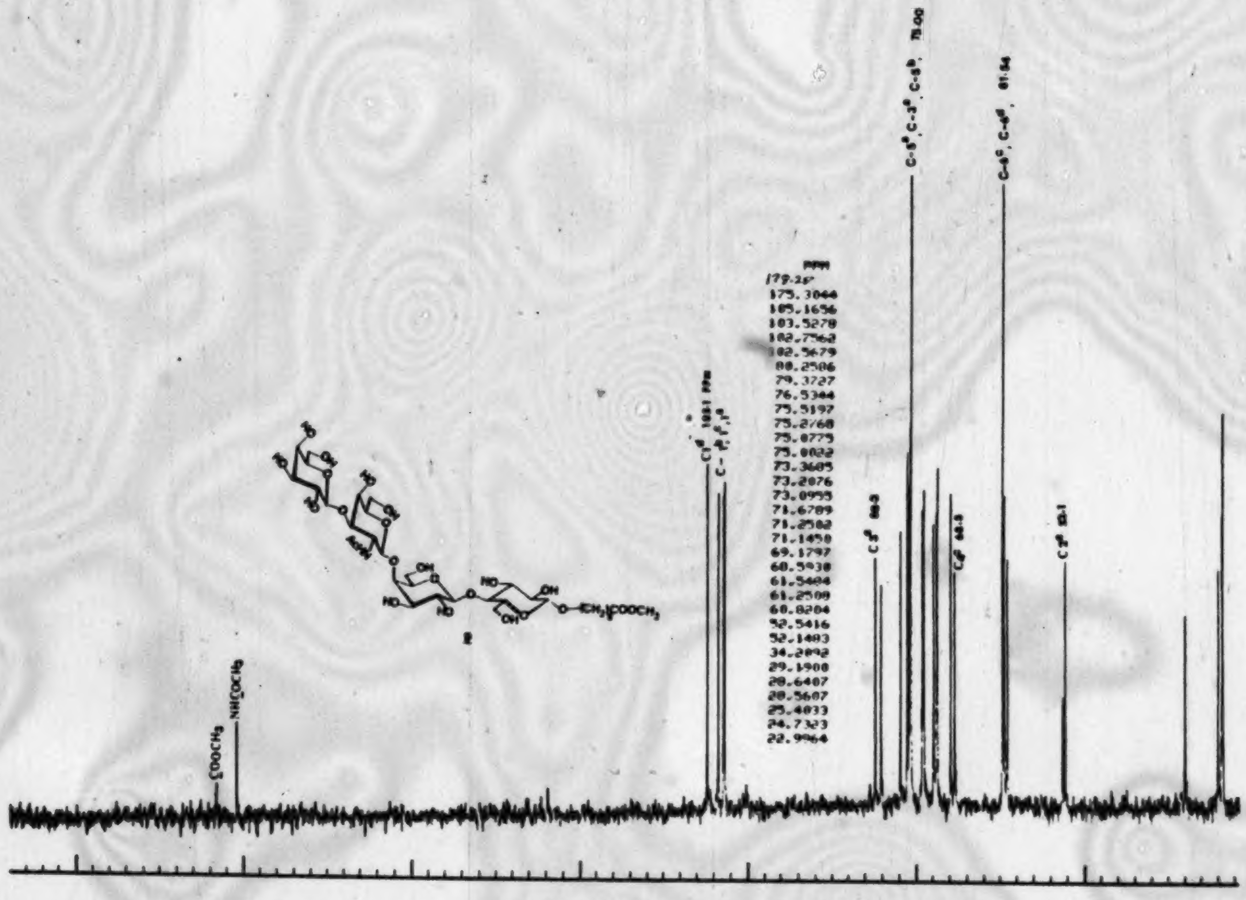


Fig.38 100 MHz ^{13}C-nmr spectrum of compound 9 in 100% deuterium oxide.

B. Conformational Analysis of the Oligosaccharide Portions of Gangliosides G_{M2} and G_{M1}

Having synthesized the oligosaccharide portions of asialo-G_{M2} and asialo-G_{M1}, it was possible to subject these compounds (8 and 9) to detailed analysis by ^{1}H- and ^{13}C-nmr. In order to assist in the interpretation of data, compounds 48, 51, 52, 16, 50* and 49 were also examined. It is seen that compounds 16, 50 and 49 contain the three disaccharide units that are present in the tetrasaccharide 9. The conclusion reached as to the most probable conformations for 8 and 9 will then be applied in an attempt to assess the conformational preferences for the related gangliosides. Throughout this section the sugar units present in 9 will be labelled a, b, c and d (terminal βDGal) and these designations will be maintained for the comparable unit in the more simple model compounds. It is expected that this procedure will help the reader to appreciate the interpretation of the nmr data.

1. The Assignment of the ^{1}H-nmr Chemical Shifts

The chemical shifts and coupling constants that could be determined for the compounds are presented in Table 4. The spectra for the oligosaccharides are

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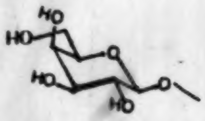
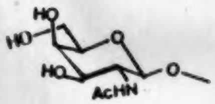
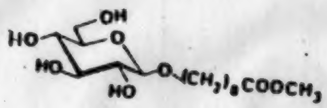
* This compound was kindly provided by Dr. R. M. Ratcliffe, Chembiomed Ltd., University of Alberta.



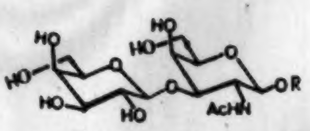
51



52

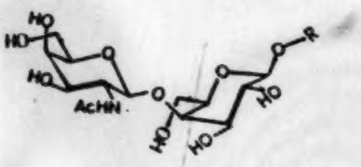


48



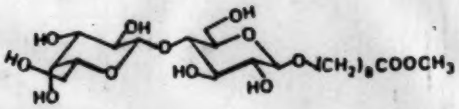
R= $-CH_2CH_2CH_3$:

49

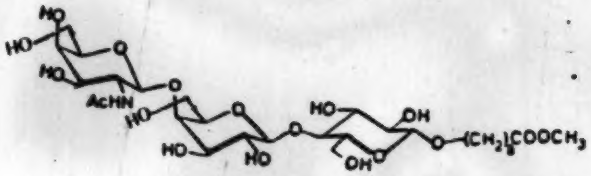


R= $-(CH_2)_8COOCH_3$.

50



16



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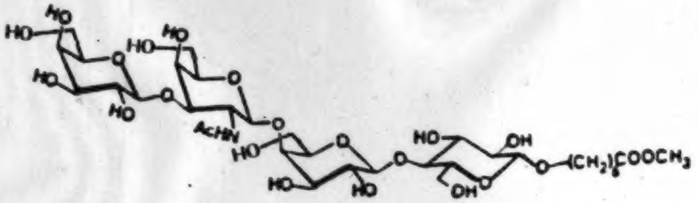

9

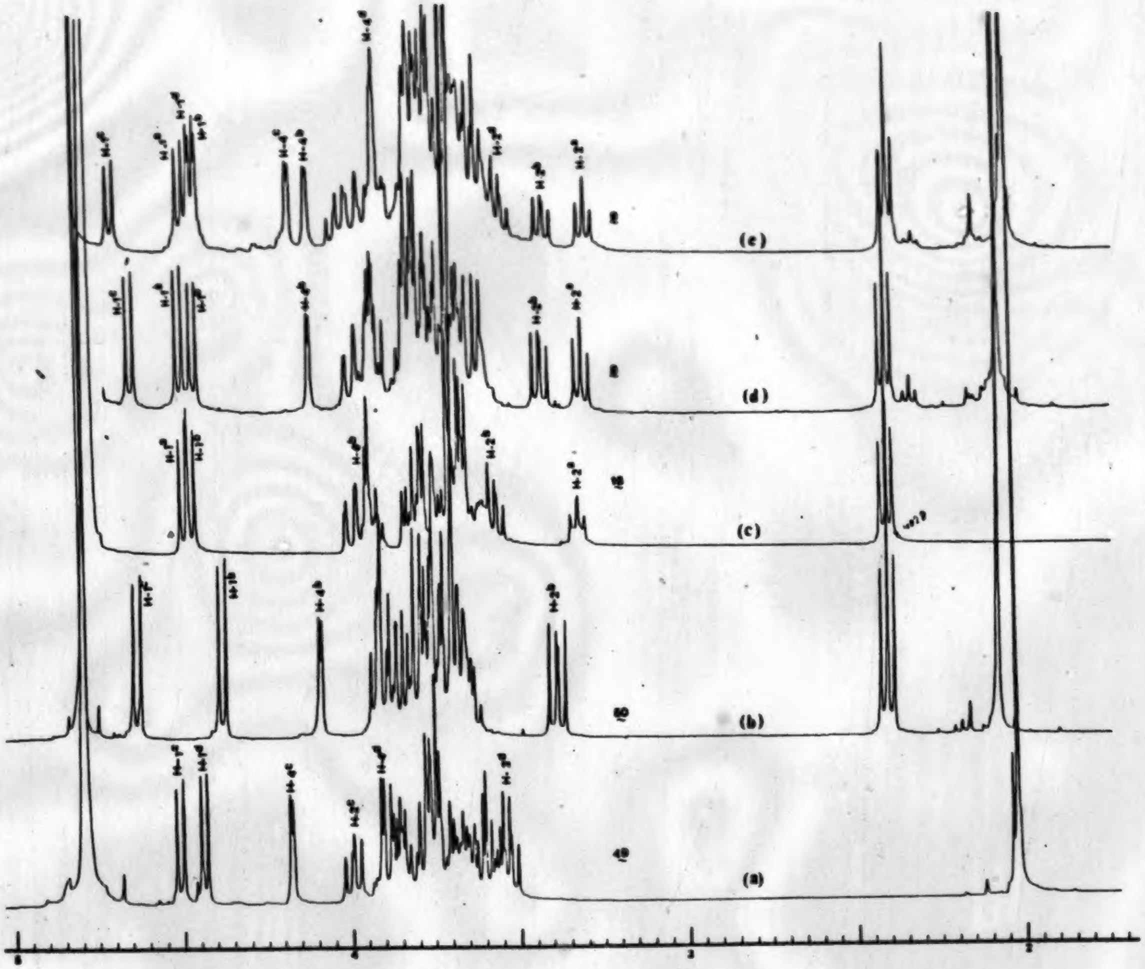

Fig. 36 Comparison of 400 MHz ^{1}H-nmr spectra of Asialo-G_{M2} and Asialo-G_{M1} related haptens 8 and 9 with those of the model disaccharides 49, 50 and 16.

TABLE 4

Comparison of ^{1}H-nmr Chemical Shifts* and Coupling Constants** (in paranthesis) of Some of the Hydrogens of the Oligosaccharide Portions of Asialo-G_{M2} and Asialo-G_{M1} Related Haptens, βDGalNAc(1→4)βDGal(1→4)βDGlc-O$(CH_2)_8$-$COOCH_3$ (8) and βDGal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlc-O$(CH_2)_8COOCH_3$ (9) with Those of the Model Compounds βDGlc-O$(CH_2)_8COOCH_3$ (48), βDGal-OCH_3 (51), βDGalNAc-OCH_3 (52), βDGal(1→4)βDGlc-O$(CH_2)_8COOCH_3$ (16), βDGalNAc(1→4)βDGal-O$(CH_2)_8COOCH_3$ (50) and βDGal(1→3)βDGalNAc-$OCH_2CH_2CH_3$ (49).

Sugar Unit	Hydrogen atom	48	51	52	16	50	49	8	9
βDGlc	H-1^a	4.45 (8.3)			4.43 (8.0)			4.43 (7.7)	4.43 (7.7)
	H-2^a	3.26 (9.6)			3.25			3.24 (8.5)	3.24 (8.2)
βDGal	H-1^b		4.31 (7.8)		4.40 (7.7)	4.32 (8.2)		4.39 (7.7)	4.39 (8.2)
	H-2^b		3.49 (9.7)		3.49 (9.9)	3.33 (9.9)		3.36 (9.9)	3.35 (10.1)
	H-4^b		3.91 (3.5)		3.88	4.03 (2.2)		4.04 (2.2)	4.05 (2.2)
βDGalNAc	H-1^c			4.38 (8.6)		4.59 (8.5)	4.53 (10.0)	4.58 (9.0)	4.64 (8.2)
	H-2^c			3.88 (10.9)		3.85	4.01 (11.2)	3.89	3.95
	H-4^c			3.93 (3.4)		3.85	4.19 (3.3)	3.88	4.11 (2.2)
βDGal	H-1^d		4.31 (7.8)				4.46 (7.7)		4.40 (7.7)
	H-2^d		3.49 (9.7)				3.54 (10.9)		3.47
	H-4^d		3.91 (3.5)				3.93 (3.3)		3.85

---

* Measured at 400 MHz in D_2O at 296°K relative to HOD (4.81 ppm).

**Observed first order coupling constants.

reproduced in Fig. 36, where it is seen that few of the signals could be assigned directly since the spectra contain large envelopes of signals that were not resolved at 400 MHz. The assignments shown were derived as follows.

The ^{1}H-nmr spectrum of the lactoside 16 was poorly resolved. The signals for the two anomeric hydrogens (H-1^{a} and H-1^{b}) occurred around 4.4 ppm as a triplet with spacings of about 8 Hz. The chemical shifts were 4.43 and 4.41 ppm, respectively. The signal at 4.43 ppm could be assigned to H-1^{a} since this signal collapsed on spin-decoupling of the multiplets at 3.25 ppm. That the latter multiplets were that of H-2^{a} was established on the basis of its chemical shift comparison with H-2 of 48. This assignment was firmly confirmed by the observation that in the ^{13}C-nmr spectrum of 16, the signal for C-2^{a} at 73.14 ppm[73,105] was enhanced on selective saturation of signals for H-2^{a}. The appearance of complex multiplets for H-2^{a} in the ^{1}H-nmr spectrum of 16 (Fig. 36c) indicated that the virtual coupling between H-2^{a} and H-4^{a} was probably due to the overlapping of the chemical shifts[106,107] of H-3^{a} and H-4^{a}. The situation was further complicated as a result of the overlapping of the signals for H-5^{a} and H-3^{b} with those of H-4^{a} and H-3^{a}. This made it virtually impossible to measure both the chemical shift and the coupling of H-4^a in order to ascertain the $^4C_1$ conformation of the pyranose ring 'a'. But evidence in support of the above $^4C_1$ conformation of the two pyranose rings of β-lactoside was elegantly obtained by Barker et al.[107] by three bond ^1H-^1H and two and three bond ^{13}C-^1H coupling constants of methyl β-D-lactoside at 600 MHz. In fact, the reported[107] chemical shifts for H-3^a and H-4^a are in accord with our predictions based on the complex pattern of signals observed for H-2^a.

The signals for the two anomeric hydrogens (H-1^b and H-1^c) of compound 50 are well resolved. It was readily possible to assign the signals for H-2^b and H-2^c by spin decoupling. The higher field quartet (J = 9.9 and 8.2 Hz) could be assigned to H-2^b on the basis of the chemical shift of the H-2 atoms for the two simple glycosides 51 and 52. This assignment of the signal for H-1^b appeared unequivocal. Nevertheless, the assignment was confirmed by saturation of the signal for H-1^c which caused a nuclear Overhauser enhancement of H-4^b (Fig. 37c).

In the case of the disaccharide unit of 49, as seen in Fig. 36a, the signals were well resolved but the separation was not as large as for 50. Spin decoupling of the signal at 4.48 ppm partially collapsed the multiplet at 3.54 ppm. Therefore, the signal at 4.48 ppm must arise from the anomeric hydrogen that is involved in the intersugar glycosidic bond and therefore assignable to H-1^d. The assignment of the signal at 3.54 ppm to H-2^d of the βDGal unit is in

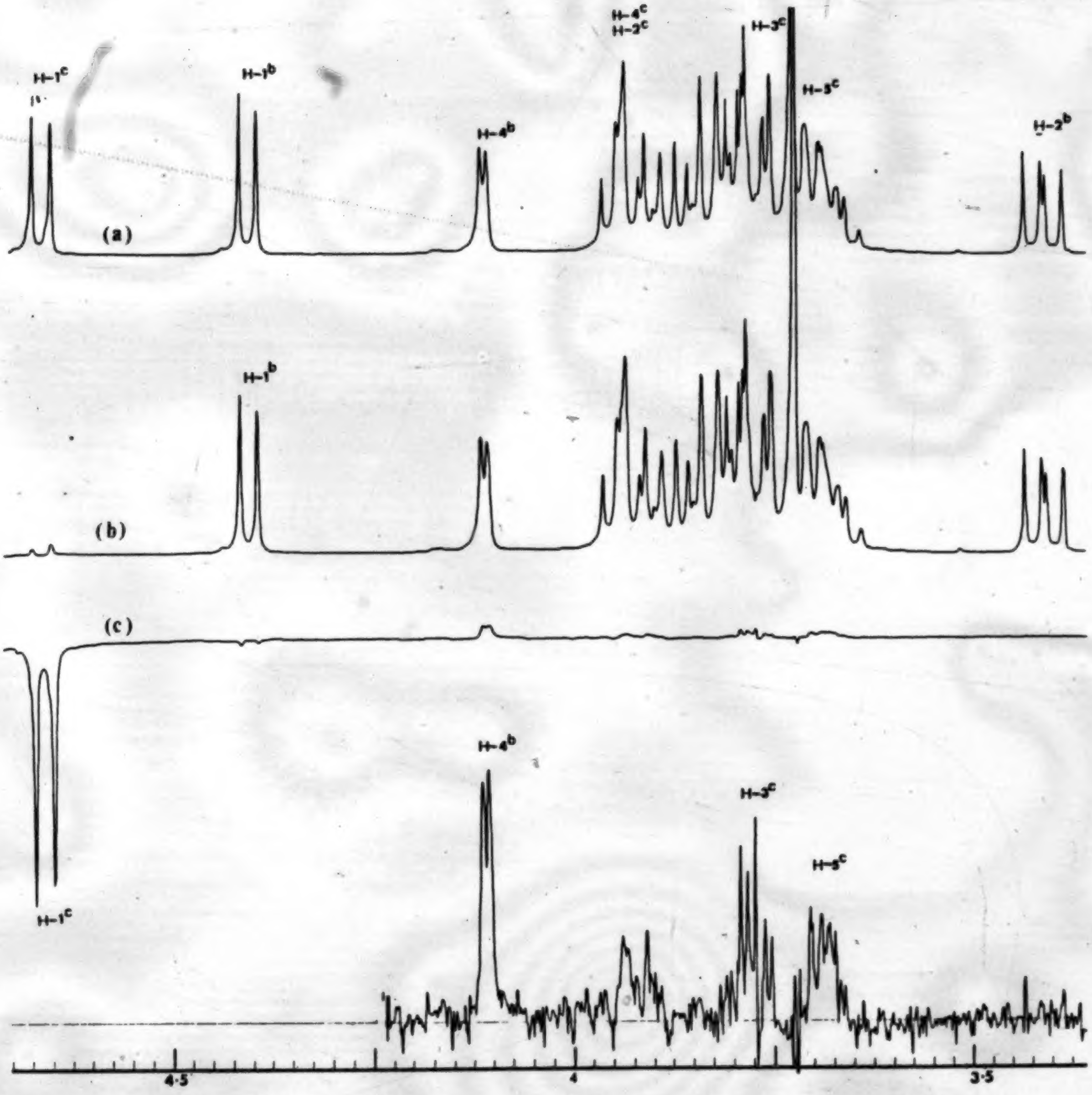

Fig. 37 The nOe enhancements observed as a result of irradiating H-1^{c} in the 360 MHz ^{1}H-nmr spectrum of compound 50: (a) the normal spectrum, (b) the iraddiated spectrum, and (c) the nOe difference spectrum.

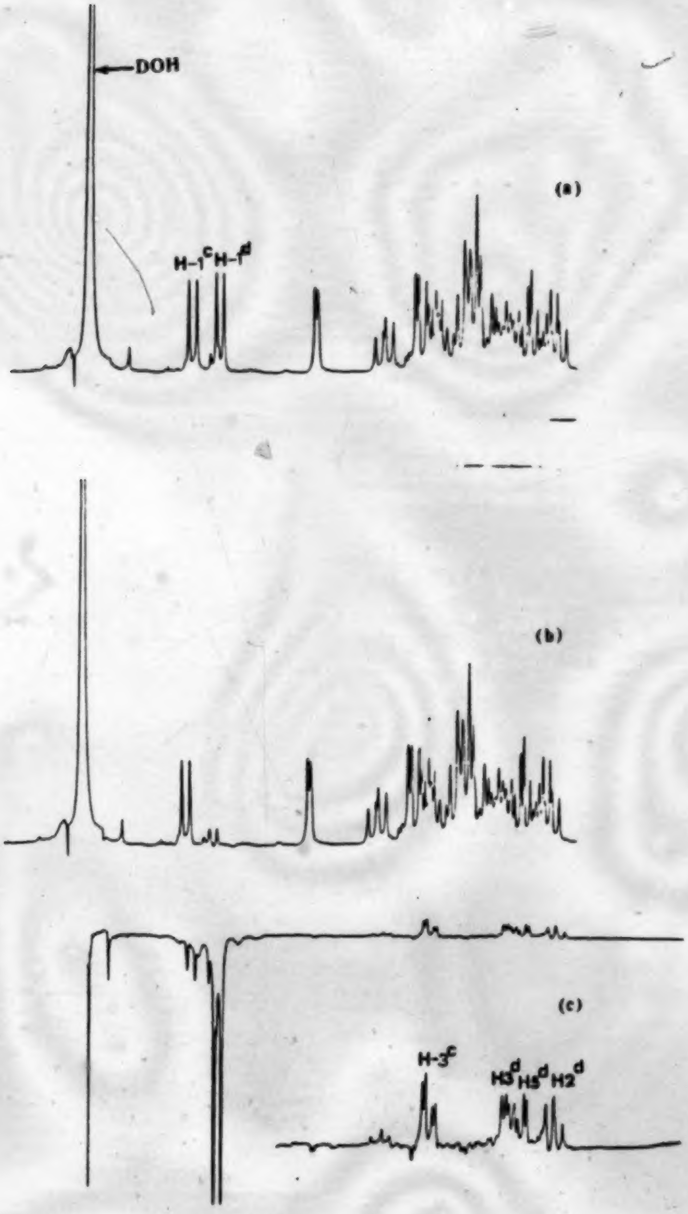

Fig. 38 The nOe enhancements observed as a result of irradiating H-1' in the 400 MHz spectrum of compound 49 in D_2O: (a) the normal spectrum, (b) the irradiated spectrum, and (c) the nOe difference spectrum.

accord with the expectation based on the chemical shifts for the H-2 atoms of **51** and **52**. Saturation of H-1^{d} caused enhancement of H-3^{d} (J = 3.0 and 10.5 Hz) and H-5^{d} (Fig. 38) as well as H-3^{c} and thereby provided their chemical shifts. Again, the vicinal coupling constants confirmed the expected $^{4}C_{1}$ conformation for the pyranose rings.

The above assignments for compounds **16**, **50** and **49** were then used to assist in the assignment of the corresponding signals for the more complex haptens **8** and **9**.

The signals for all three anomeric hydrogens of asialo-G_{M2} hapten (**8**) were well resolved in the ^{1}H-nmr spectrum (Fig. 36d) and therefore could be readily assigned on the basis of comparison with those of **50** and **16** (Table 4). Thus, the signal at 4.58 ppm (J = 9.0 Hz) was assigned to H-1^{c} and this was also confirmed by spin-decoupling experiments, where the saturation of the signal for this hydrogen partially collapsed the multiplets around 3.88 ppm. Since these latter signals were at positions typical for H-2^{c} of the βDGalNAc residue, the above assignment for H-1^{c} was established. The chemical shifts of the remaining two anomeric hydrogens were established by comparison of their chemical shifts with the corresponding shifts in **16** and **50** and then further confirmed by spin-decoupling experiments of the high field multiplets at 3.36 ppm (H-2^{b}, J = 9.9 and 7.7 Hz) and 3.24 ppm (H-2^{a}, J = 7.7 and 8.5 Hz).

Thus, the chemical shifts for H-1^a and H-1^b were determined as 4.43 (J = 7.7 Hz) and 4.39 (J = 7.7 Hz) ppm, respectively. The only other signal that was well resolved, and that could be readily assigned, was that of H-4^b (4.04 ppm) on the basis of comparison of its chemical shift in 50. Thus, the similar chemical shifts and coupling constants for H-1^c, H-2^c, H-1^b, H-2^b, H-4^b, H-1^a and H-2^a (Table 4) in compounds 8, 50 and 16 strongly suggested that indeed the $^4C_1$ conformation was maintained in 8 also.

In contrast to compound 8, the ^{1}H-nmr spectrum of *asialo*-G_{M1} hapten (9) (Fig. 36e) was more complex but still the chemical shifts of the anomeric hydrogens could be determined with confidence by simple comparison of the ^{1}H-nmr spectrum of 9 with those of 8 and 49. These are presented in Table 4. Also, the weakly coupled equatorial hydrogens of the βDGalNAc (H-4^c) and the inner βDGal (H-4^b) units could be assigned to signals at 4.11 and 4.05 ppm, respectively. As expected, the high field multiplets at 3.35 ppm (H-2^b) and 3.24 ppm (H-2^a) in compound 8 were not affected as a result of glycosylation at 3-OH^c to form 9. These remarkable similarities of chemical shifts among compounds 9, 8 and 49 led to the conclusion that all pyranose rings in the tetrasaccharide 9 reside predominantly in the $^4C_1$ conformation.

2. The Assigments of ^{13}C-nmr Chemical Shifts

The ^{13}C-nmr chemical shifts for the model compounds 48, 51, 52, 49, 50, 16, 8 and 9 are presented in Table 5. It can be seen that the resolution at 100 MHz was sufficient to allow, in each case, the assignment of a line for every carbon in the molecule. In the case of the tetrasaccharide 9, signals were not well resolved at 73.09 and 75.00 ppm (Fig. 35). Nevertheless, the coincidence was evident from the intensities of these signals relative to those for other secondary carbon atoms.

The assignments of signals in Table 5 were all done by comparison. It is realized that this procedure may lead to errors. However, such errors are expected to involve shifts that differ by less than 0.2 ppm and are inconsequential to the conformational analysis. For example, the signal at 75.00 ppm, which is assigned in Table 5 to C-5^{a} of 9, could in fact arise from C-5^{c} which is assigned to the signal at 75.07 ppm. Although an extensive nmr examination may have allowed unequivocal assignments of all the signals, such an expensive exercise did not seem warranted for the purpose of this thesis since any such errors would not affect the conclusions to be drawn from the data.

It is well established[108] that the glycosylation of a sugar hydroxyl group to form a disaccharide normally leads

TABLE 5

The Assigments of the ^{13}C-Chemical Shifts* for the Asialo-G_{M2} and Asialo-G_{M1} related haptens βDGalNAc(1→4)βDGal(1→4)βDGlcO$(CH_2)_8COOCH_3$ (**8**) and βD-Gal(1→3)βDGalNAc(1→4)βDGal(1→4)βDGlcO$(CH_2)_8COOCH_3$ (**9**) based on the Shifts Observed for the Model Compounds βDGlc-O$(CH_2)_8COOCH_3$ (**48**), βDGalOCH$_3$ (**51**), βDGalNAcOCH$_3$ (**52**), βDGal(1→4)βDGlcO$(CH_2)_8COOCH_3$ (**16**), βDGalNAc(1→4)βDGalO-$(CH_2)_8COOCH_3$ (**50**) and βDGal(1→3)βDGalNAcO$(CH_2)_2CH_3$ (**49**).

Sugar Unit	Carbon Atom	Chemical Shifts, ppm 51	52	48	16	50	49	8	9
βDGlc	1a			102.54	102.31			102.37	102.56
	2a			73.40	73.14			73.16	73.36
	3a			76.14	74.76			74.76	75.00
	4a			69.88	78.81			79.04	79.37
	5a			76.14	75.02			75.10	75.07
	6a			61.06	60.45			60.53	60.81
βDGal	1b	104.08			103.22	102.80		103.34	103.52
	2b	71.00			71.25	71.16		71.45	71.20
	3b	73.05			72.85	73.08		72.90	73.09
	4b	68.94			68.84	76.15		76.48	76.53
	5b	75.36			75.62	74.29		74.78	75.00
	6b	61.23			61.27	60.61		61.03	61.24
βDGalNAc	1c		102.63			102.98	101.56	102.94	102.75
	2c		52.54			52.99	51.63	53.08	52.14
	3c		71.39			71.26	80.19	71.45	80.25
	4c		68.10			68.12	68.32	68.21	68.59
	5c		75.33			75.09	75.00	75.21	75.27
	6c		61.22			61.33	61.19	61.41	61.54
βDGal	1d	104.08					105.06		105.16
	2d	71.00					70.92		71.14
	3d	73.05					72.78		73.09
	4d	68.94					68.88		69.17
	5d	75.36					75.26		75.51
	6d	61.23					61.28		61.54

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* Measured at 100 MHz in D_2O at 305°K with external tetramethylsilane as reference signal (0 ppm).

to a deshielding of the new aglyconic carbon by about 7 ppm. This, however, may not be the case should the glycosylation provide a branched trisaccharide with glycosyl groups at neighboring positions.[84] Thus, the signals for the βDGal unit of 16 could be assigned from those established[96] for βDGal-O-CH_3 (Table 5). The introduction of the βDGalNAc unit on C-4^b to provide 8 would be expected to cause deshielding of this atom (68.84 ppm) by about 7 ppm and, in fact, the signal assigned to C-4^b of 8 at 76.48 ppm is in accord with this expectation. The assignments in Table 5 for units a and b were made following similar considerations. Furthermore, the chemical shifts for the βDGlc units of 16, 8 and 9 were arrived at in this way and are seen to be in good accord with expectation based on the chemical shifts for βDGlc-$O(CH_2)_8COOCH_3$. In fact, the assignments listed for 16 are in accord with those reported for the same compound by Banoub and Bundle.[73] The assignments made for the first two residues (βDGlc and βDGal) of the oligosaccharide (Table 5) are therefore made with a high level of confidence except for the signals for C-1^b and C-2^b of the disaccharide unit of compound 50. Obviously C-1^b may have occurred at 102.98 ppm and C-2^b at 71.26 ppm, the signals which are assigned to C-1^c and C-3^c of 50, respectively. The assignment of the signals for C-6^a and C-6^b of 8 and 9 are evidently provisional since there is no reason to expect that the conformational preference for a freely rotating group will remain constant.

Similar considerations then led to the assignment of the signals for the βDGalNAc unit of 8 and the βDGal(1→3)-βDGalNAc unit of 9. The correspondences of chemical shifts are evident and do not require detailed discussion. As mentioned above, although most of the assignments are likely correct, some errors may exist on the choice between two signals for secondary carbons that differ little in chemical shifts or for the hydroxymethyl groups since these may acquire somewhat different conformational equilibria as the result of the structural changes.

3. Conformational Analysis of the Haptens 8 and 9.

The good correspondence between the ^{13}C-chemical shifts for those atoms about the glycosidic linkages in compounds 8 and 9 and those for the carbon atoms about the intersugar residues in compounds 16, 50 and 49 (see Table 6) can be taken as evidence that the conformational preferences for the disaccharides are well maintained in the corresponding units present in the higher oligosaccharides. It was considered meaningful, therefore, to establish the conformational preferences for the disaccharides on the assumption that these are maintained in compounds 8 and 9. In this regard, it is

TABLE 6

The Correspondence in Chemical Shifts (ppm) Observed for Atoms that are Common in the Structures Listed (see Tables 4 and 5)

The βDGal(1→4)βDGlc Linkage:

Compound	C-1[b]	C-4[a]	H-1[b]	H-4[a]
16	103.22	78.81	4.40	--
8	103.34	79.04	4.39	--
9	103.52	79.37	4.39	--

The βDGalNAc(1→4)βDGal Linkage:

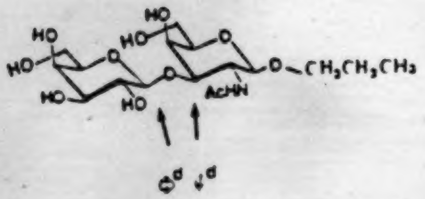
Compound	C-1[c]	C-4[b]	H-1[c]	H-4[b]
50	102.98	76.15	4.59	4.03
8	102.94	76.48	4.58	4.04
9	102.75	76.53	4.64	4.05

The βDGal(1→3)βDGalNAc Linkage:

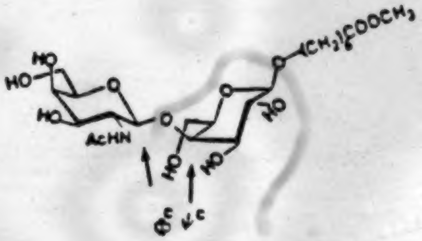
Compound	C-1[d]	C-3[c]	H-1[d]	H-3[c]
49	105.06	80.19	4.46	3.82
9	105.16	80.25	4.40	

useful to first obtain a molecular model which can then be tested by appropriate nmr experiments. Since HSEA calculations have provided useful models for this purpose,[84] this method was applied to the disaccharides 16, 50 and 49.

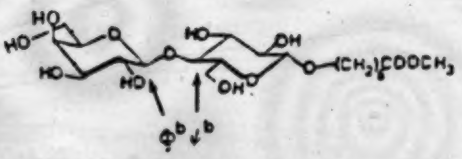
The various torsion angles ϕ^H and ψ^H for the glycosidic linkages are designated as indicated in the following formulas. The τ angle is the angle defined by the glycosidic carbon, glycosidic oxygen and aglyconic carbon atoms.


HO
HO
HO
HO
HO
HO
AcHN
O–CH2CH2CH3
ϕa ψa

49


HO
HO
HO
AcHN
O–(CH2)8COOCH3
HO
HO
HO
ϕc ψc

50


HO
HO
HO
HO
HO
OH
OH
O–(CH2)8COOCH3
ϕb ψb

16

As usual, the three-dimensional co-ordinates of the individual sugar units required to program the HSEA calculations were obtained from the published X-ray crystallographic data for methyl β-D-galactopyranoside,[109] N-acetyl-β-D-galactosamine,[110] and gentiobiose.[111] The models were built by using a τ angle of 117°[84] and by varying the ϕ^H torsion angle from +180° to -180° at intervals of 5° for each ψ^H torsion angle examined. The choice of the ψ^H values was limited to the range +20° to -20° (using 5° intervals) since it is well established[80] that the minimum energy conformers for simple β-linked disaccharides fall in this range. The minimum energy conformers are presented in Table 7 along with internuclear distances that will be relevant to the discussion of the results obtained in the nuclear Overhauser enhancement studies.

As was mentioned earlier in connection with the assigment of ^{1}H-chemical shifts, the β-lactoside was not well amenable to nOe studies based on the selective saturation of H-1^b since its signal was separated from that for H-1^a by only 0.03 ppm (2.4 Hz). In addition, the signals for H-4^a overlapped with those for H-3^b and H-5^b which would also exhibit nOe enhancements. Therefore, for the present purposes, it is assumed that the conformational preference for 16 is that estimated by HSEA calculation and the same as

TABLE 7

The Conformational Preferences for the Model Disaccharides as Estimated by HSEA Calculation

Disaccharide Unit	ϕ^H	ψ^H	Internuclear Distances, Å
βDGal(1→4)βDGlc	55°	0°	From H-1^b: 2.442* (H-4^a), 4.494 (H-3^a), 3.905 (H-5^a), 3.060 (H-2^b), 2.565 (H-3^b), 2.278 (H-5^b)
βDGalNAc(1→4)βDGal	55°	10°	From H-1^c: 2.460 (H-4^b), 3.999 (H-3^b), 4.749 (H-5^b), 3.063 (H-2^c), 2.597 (H-3^c), 2.414 (H-5^c)
βDGal(1→3)βDGalNAc	55°	10°	From H-1^d: 2.460 (H-3^c), 4.205 (H-4^c), 3.814 (H-2^c), 3.060 (H-2^d), 2.565 (H-3^d), 2.278 (H-5^d)

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* Those atoms within the van der Waals radius are underlined.

that published for the human blood group Type 2 disaccharide, βDGal(1→4)βDGlcNAc.[84] In fact, these estimated values of the torsion angles (ϕ^a,ψ^a = 55°,0°) are in good agreement with those reported by Barker et al.[107] based on ^{13}C-^{1}H three bond coupling constants.

The internuclear distances reported in Table 7 for compound 50 show H-1^c to be sufficiently close to H-4^b, H-2^c, H-3^c and H-5^c so that saturation of H-1^c should show an enhancement of the signals for these hydrogen atoms. Examination of the difference spectrum, reproduced in Fig. 37c, shows that in fact several signals were enhanced but the quality of the spectrum allowed only the assignment of the enhancements for the signals of H-4^b and H-2^c. Although the data was not suitable for further analysis, the strong enhancement (9%) observed for H-4^b is clearly in accordance with the model obtained by HSEA calculation which sets the distance between H-1^c and H-4^b at less than the sum of their van der Waals radii (2.7 Å).[112]

As seen in Fig. 38, the quality of the nOe measurements for compound 49 was excellent. Saturation of H-1^d caused enhancement of all the signals for the hydrogens that were expected to be enhanced on the basis of the HSEA calculation (Table 7); namely, H-3^c, H-2^d, H-3^d and H-5^d. The enhancements were 9% (H-3^c), 17% (H-3^d + H-5^d) and 7% (H-2^d). A quantitative assessment of these enhancements[80] was not attempted.

The fact that the nOe experiments for compounds 50 and 49 proved to be in qualitative agreement with expectation based on HSEA calculation, together with the ^{13}C-nmr chemical shift data indicating that the conformational preferences for the disaccharide units of compounds 16 and 50 are maintained in compound 8 and those for 16, 50 and 49 are maintained in compound 9, suggest that the energetically most favored conformers for the oligosaccharide portions of 8 and 9 are near those represented by computer-drawn projections of CPK models in Fig. 39 and 41 and of Dreiding models in Fig. 40 and 42. The main purpose of these drawings is to allow the reader to accurately construct the models predicted by HSEA calculation. Furthermore, a consideration of these drawings does provide an appreciation of the differences in the shapes of the molecules and how different views offer different topographies for binding of a lectin or antibody combining site.

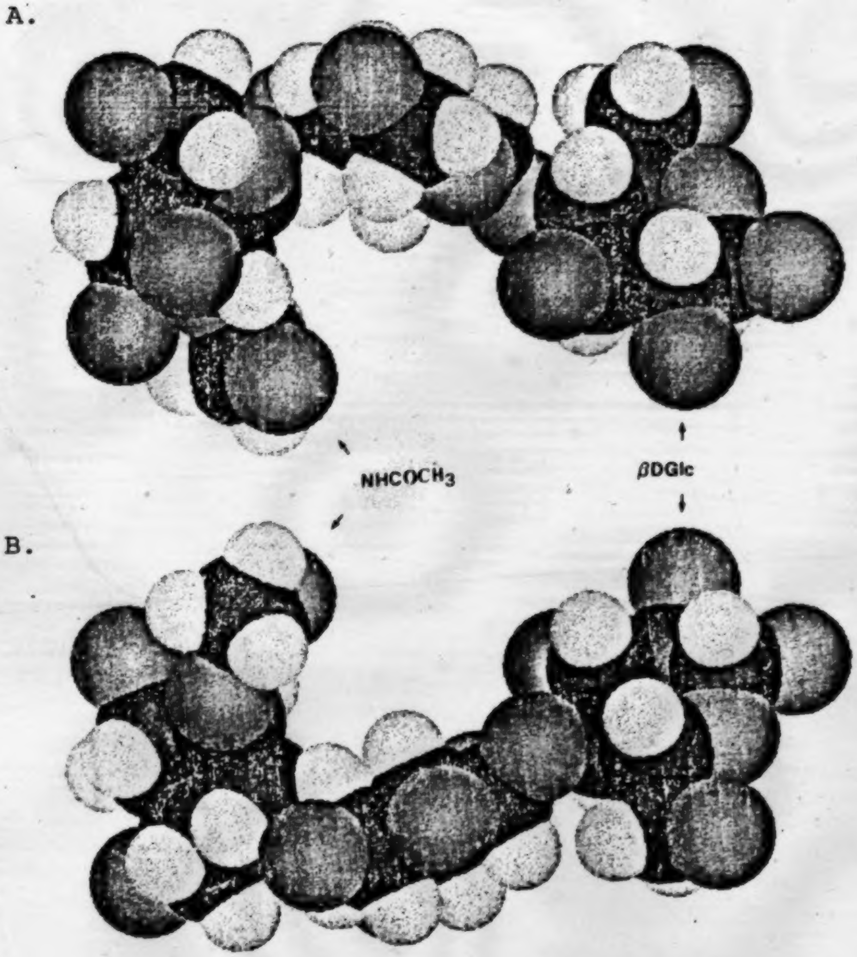


Fig. 39 The computer drawn projections of the CPK model of the oligosaccharide portion of the hapten 8 and of asialo-G_{M2} as derived by HSEA calculation:
A. the molecule as viewed along H-2^c - C-2^c bond;
B. the view of the side of the molecule opposite to that in A.

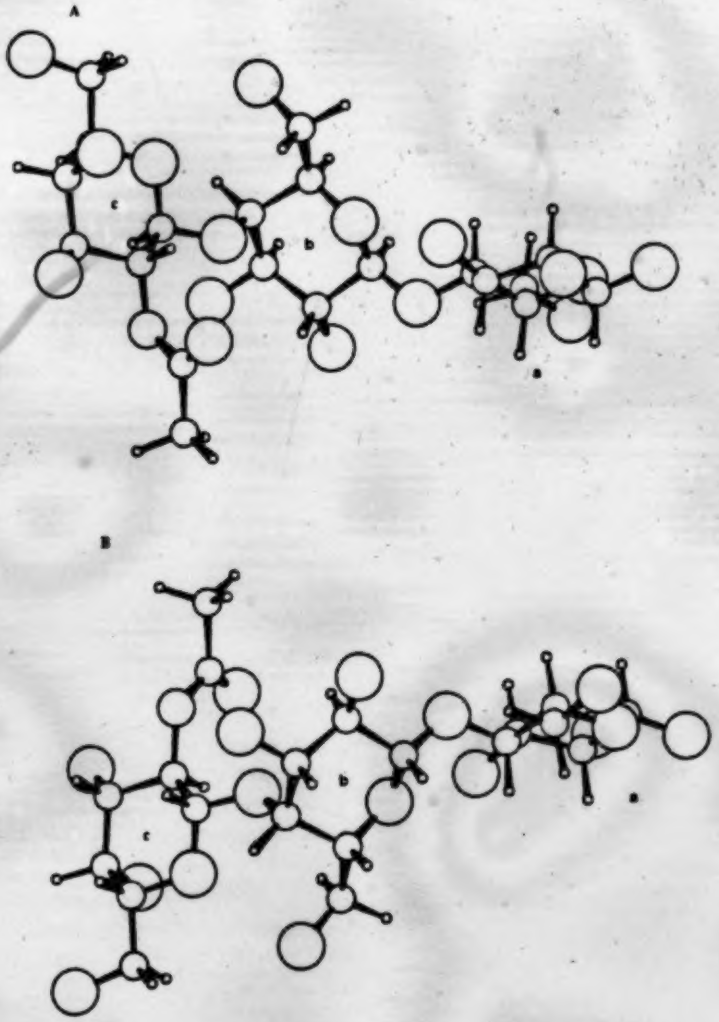


Fig.40 . The computer drawn projections of the Drieding model of the oligosaccharide portion of the hapten **8** and asialo-G_{M2} as derived by HSEA calculation: A. the molecule as viewed along H-2^{c} - C-2^{c} bond; B. the view of the side of the molecule opposite to that in A.

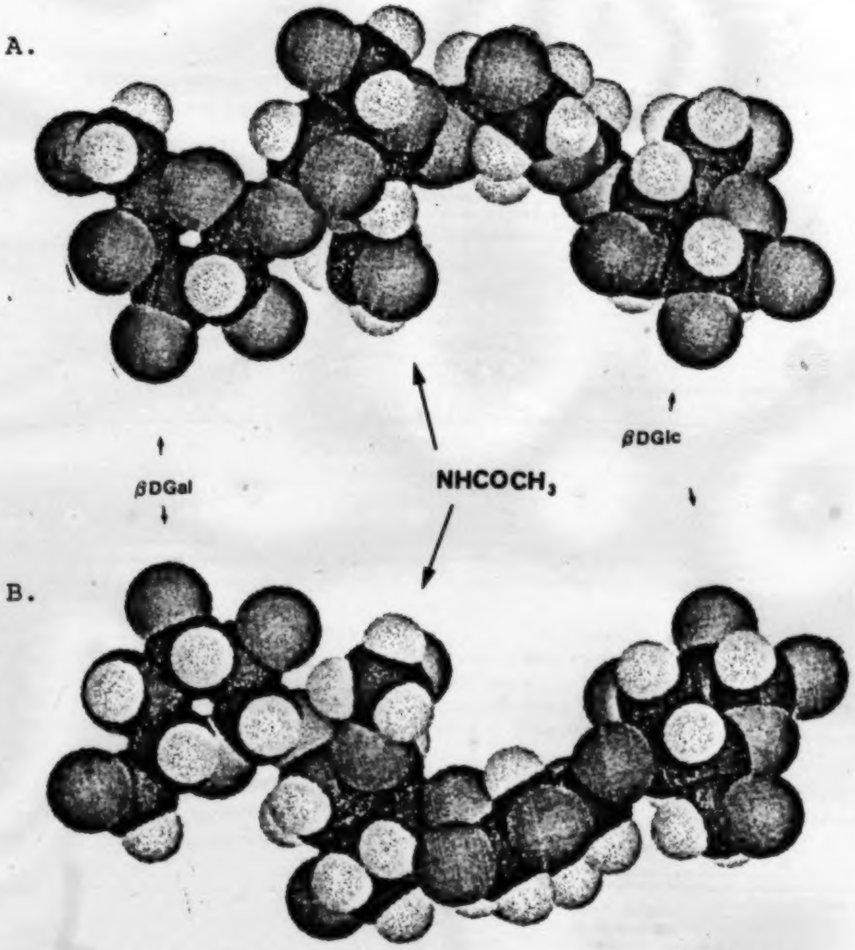


Fig.41 . The computer drawn projections of the CPK model of the oligosaccharide portion of asialo-G_{M1} as derived by HSEA calculation. A. the view of the molecule along H-2^c - C-2^c bond; B. the view of the side of the molecule opposite to that of A.

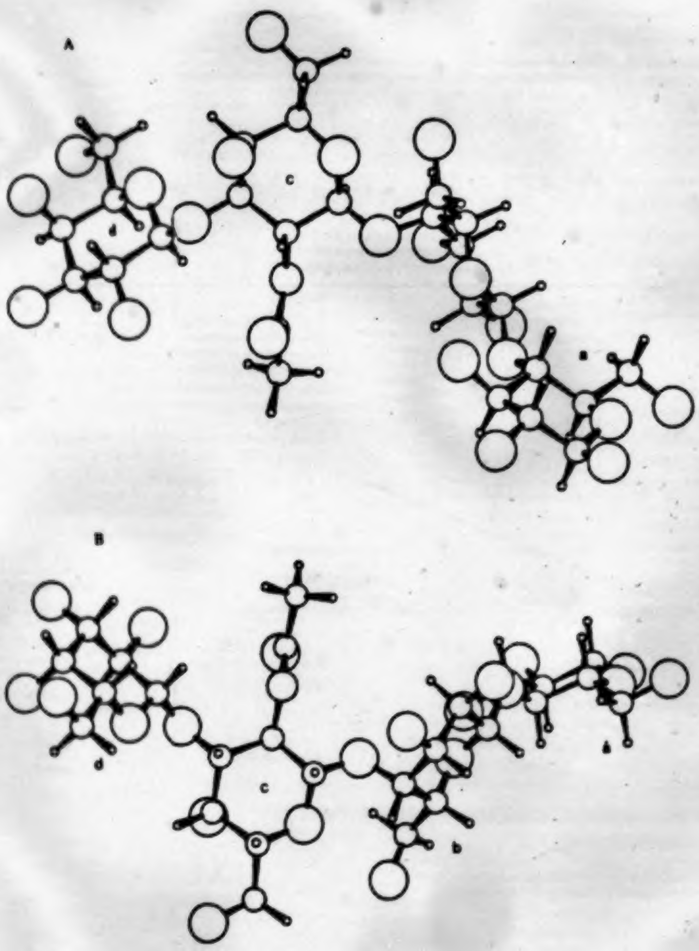
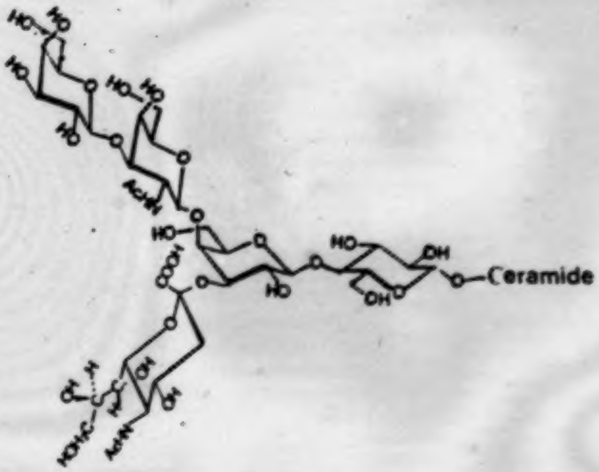


Fig.42 The computer drawn projections of the Drieding model of the oligosaccharide portion of asialo-G_{M1} as derived by HSEA calculation: A. the view of the molecule along H-2^c - C-2^c bond; B. the view of the side of the molecule opposite to that of A.

4. Conformations of the Oligosaccharide Portions of the Ganglioside G_{M1}

The conformational analysis of oligosaccharide structures has advanced sufficiently in recent years[84] to allow the presentation of the pentasaccharide unit of G_{M1} as shown in the conformational formula **53**. However, such two-dimensional formulas provide only a very approximate appreciation of the molecular structure and are of very limited use to immunochemistry. This section is concerned with an attempt to achieve a three-dimensional model for G_{M1} in its minimum energy conformation.



53

Fig. 43 The approximate conformation of the oligosaccharide portion of the ganglioside G_{M1}.

A synthetic sample of the methyl glycoside of the sodium salt of N-acetyl-α-D-neuraminic acid (10) was prepared[113] and examined by ^{13}C-nmr. The chemical shifts reported in Table 8 correspond exactly with the literature values except by a displacement of 1.0 ppm.[114]

An authentic sample of G_{M1} was available* in an amount adequate for ^{13}C-nmr spectroscopy. The chemical shifts are reproduced in Table 8 along with those obtained for hapten 9. In order to assess whether the introduction of the α-NeuNAc group at O-3^b of asialo-G_{M1} caused a change in the conformational equilibrium of the neutral core oligosaccharide, an attempt was made to assign the signals for G_{M1} (53) on the basis of the signals observed for methyl αDNeuNAc (10) and the asialo-G_{M1} related hapten 9. To this end, the first step was to extract the signals from the spectrum for G_{M1} that correspond best to those found for methyl α-sialoside (10) except for the anomeric carbon where the shift cannot be expected to be the same because of the change in aglycon. One of the two signals that occurred at 102.92 ppm was used for this purpose. Since it is considered reasonable to

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*Kindly donated by Dr. S.K. Kundu, Dept. of Internal Medicine, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030

TABLE 8

The Provisional Assigment of the ^{13}C-nmr Chemical Shifts for the Ganglioside G_{M1} on the Basis of the Assignment for the Sodium Salt of αDNeuNAc (10) and Hapten 9 (Table 5) and the Comparison of the Assignment with that Published for G_{M1}*

Sugar Unit	Carbon Atoms	Sodium Salt of Me-α-D-NeuNAC 10	9	GM_1	G_{M1}-9	G_{M1}*	δG_{M1}-δG_{M1}*
βDGlc	1a		102.56	102.24	-0.32	103.40	-1.16
	2a		73.36	73.56	0.20	74.09	-0.53
	3a		75.00	74.75	-0.25	75.23	-0.48
	4a		79.37	79.41	0.04	79.80	-0.39
	5a		75.27	75.23	-0.04	75.74	0.51
	6a		60.81	60.71	0.10	61.00	-0.29
βDGal	1b		103.52	103.20	-0.32	103.40	-0.20
	2b		71.20	70.31	0.11	70.73	-0.42
	3b		73.20	77.93	4.73	75.23	2.70
	4b		76.53	74.75	-1.78	78.14	-3.39
	5b		75.00	74.75	0.25	75.00	-0.25
	6b		61.24	61.05	-0.19	61.83	-0.78
βDGalNAc	1c		102.75	102.92	0.17	103.40	-0.48
	2c		52.14	51.50	-0.64	52.77	-1.27
	3c		80.25	80.90	0.65	81.52	-0.42
	4c		68.59	68.92	0.33	68.75	0.17
	5c		75.07	75.23	0.16	75.74	-0.51
	6c		61.54	61.37	-0.17	61.43	-0.06
βDGal	1d		105.16	105.07	-0.09	105.62	-0.55
	2d		71.14	71.12	-0.02	71.89	-0.77
	3d		73.09	73.18	0.09	73.59	-0.41
	4d		69.17	69.69	0.52	69.20	0.49
	5d		75.51	75.23	-0.28	75.74	-0.51
	6d		61.54	61.37	-0.17	61.43	-0.06
Sodium salt of αDNeuNAc	1e	173.67		174.46		174.53	-0.07
	2e	100.99		102.92		102.51	0.41
	3e	40.38		36.77		37.76	-0.99
	4e	68.56		68.52		69.20	-0.68
	5e	52.21		52.21		51.96	0.25
	6e	72.86		72.99		73.59	-0.60
	7e	68.49		68.30		69.20	-0.90
	8e	71.94		72.55		72.86	-0.31
	9e	62.94		63.34		63.98	-0.64

---

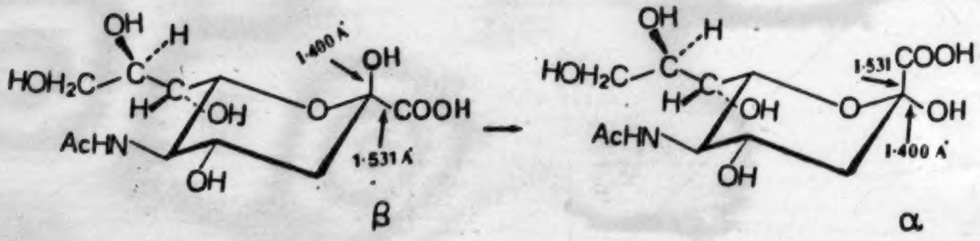
*Measured under the conditions described in Table 5 except for the published values[116] which were measured at 67.89 MHz in a mixture of D_2O-CD_3OD (1:1; v/v) at 42°C under buffered conditions (pH = 7.1).

expect that the unit that was least likely to be affected by introduction of the sialosyl group would be the terminal βDGal unit, chemical shifts were then extracted from the spectrum of 9 to match those in the spectrum of G_{M1}. The next unit expected to be least influenced would be the βDGlc group and, accordingly, these signals were then assigned. Because of the effect on chemical shifts that would be made by the substitution of the βDGal (unit b), the chemical shifts for this unit could not be anticipated on the basis of the available data. Therefore, the signals for the βDGalNAc unit of G_{M1} were assigned from the group of unassigned signals that remained. As it turned out, the same assignments would have been made in the absence of this consideration.

Assignments of the ^{13}C-chemical shifts for G_{M1} have already been published by Sillerud et al.[116,117] These assignments are presented in Table 8 where these are compared with those presently made. Substantial agreement exists in spite of the fact that the spectra were evidently not measured under comparable conditions. For example, there can be no doubt that the signals for C-2^{c} and C-5^{e} are correctly identified but a substantial discrepancy occurs regardless of how these are assigned.

On the basis of the presently assigned chemical shifts for G_{M1}, as seen from the column for G_{M1}- **9**, the chemical shift values for most of the carbons are in excellent agreement with expectations. The introduction of the α-sialosyl group at C-3^{b}, as found in G_{M1} (**53**), has profound effects on the chemical shifts for C-2^{b}. C-3^{b} and C-4^{b}. The deshielding of 4.73 ppm for C-3^{b} in **54** as compared to **9** is lower then normally observed (7-8 ppm) for linear oligosaccharide structures.[108] This is not surprising since Lemieux et al[84] have demonstrated that the glycosylation of the position vicinal to the existing glycosidic linkage (e.g.; C-3^{b} and C-4^{b} in **9**) to provide a branched structure results in the deshielding of the carbon involved in the newly formed glycosidic linkage (C-3^{b} of **54**) by about half the value of that normally seen in linear structures, while an enhanced shielding is observed for the adjacent carbon (C-4^{b} of **54**) which is already glycosidated. In fact, it can be readily seen from Table 8 that the shielding observed for C-4^{b} (1.78 ppm) is twice that observed for C-2^{b} (0.89 ppm) on going from **9** to **54**. However, no such rationalization could be provided for the changes in ^{13}C-chemical shifts of C-3^{c} (0.75 ppm), C-2^{c} (-0.64 ppm) and C-4^{d} (0.52 ppm) and it is provisionally accepted that the substitution of the α-sialosyl group at C-3^{b} caused a specific deshielding or shielding of these carbon atoms.

Regardless of the overall correctness of the assignments made in Table 8, the relatively small $\delta G_{M1}-\delta 9$ values obtained for C-1[b] (-0.32), C-1[c] (0.17) and C-1[d] (-0.09) appear correct and on this basis it can be expected that the conformational preference for the oligosaccharide unit of *asialo*-G_{M1} is essentially maintained when this compound is converted to G_{M1}. In an attempt to gain further information on this matter, an HSEA calculation was made for the trisaccharide unit βDGalNAc(1→4)[αDNeuNAc(2→3)]βDGal of G_{M1} for this purpose. The co-ordinates for the atoms of the NeuNAc residue were obtained from the crystallographic data provided by Flippen[115] for βDNeuNAc acid (2) but exchanging the positions of the carboxyl group (C-1) and the O-2 atom as shown below.


OH
H
HOH₂C
C
C
H
OH
O
AcHN
OH
1.400 Å
OH
COOH
1.531 Å
β
OH
H
HOH₂C
C
C
H
OH
O
AcHN
OH
1.531
COOH
OH
1.400 Å
α

2

55

The carboxyl group was oriented in the direction which provided, as estimated by hard-sphere calculation, the least non-bonded interaction with O-2, O-6 and H-3 (equatorial). This procedure estimated torsion angles for the C^1-O^a $(\overset{|}{\underset{|}{\mathrm{C}}}{=}\mathrm{O})$ and C^1-O^b bonds with the C^2-O^2 bond to be -168° and +12°, respectively. On this basis, the HSEA calculations were performed with the carboxyl group in this orientation following the iterative procedure used by Thøgersen et al.[80] to estimate the minimum energy conformation for the human B blood group trisaccharide (αLFuc(1→2)-[αDGal(1→3)]βDGal.

TABLE 9

The Conformational Preferences for the Four Disaccharide Units Present in Ganglioside G_{M1} (**54**) as Estimated by HSEA Calculation

Disaccharide Unit	ϕ^H	ψ^H
βDGal(1→4)βDGlc	55°	0°
βDGalNAc(1→4)βDGal	55°	10°
βDGal(1→3)βDGalNAc	55°	10°
αDNeuNAc(2→3)βDGal	-165°	10°

The calculations showed (see Table 9) that the preferred conformer well maintains the ϕ and ψ torsion angles calculated for the tetrasaccharide unit of asialo-G_{M1}. This observation lends support to the conclusion drawn from the ^{13}C-chemical shifts data (Table 8).

On the basis of the HSEA molecular model (Fig. 41 Fig. 42) for asialo-G_{M1}, as seen in Fig. 44, one side of the molecule (A) is strongly hydrophilic in the sense that it is rich in oxygen atoms and hydroxyl groups. In contrast, the other side (B) has an extensive topography that can be expected to be compatible with a hydrophobic surface. The achievement of this insight is of major interest since the work of this laboratory has demonstrated,[79,118,119] in recent years, that there exists a strong hydrophobic bonding component to the specific binding of oligosaccharides by antibodies and lectins. The corollary of these findings is that the lipophilic regions of the topography of an oligosaccharide antigenic determinant is importantly involved in dominating the immune response. Evidence in support of this contention has been obtained,[79] and the subject undoubtedly will become of major interest as a tool in the general area of immunology in the sense that it already appears predictable that the projection shown in Fig. 41B for asialo-G_{M1} will be immunodominant. It is to be noted in this regard that the conversion of asialo-G_{M1} to the ganglioside G_{M1} involves the insertion of the α-sialosyl group on this hydrophobic side of the molecule with the carboxyl group occupying a central position. Thus, it appears predictable that antibodies specific for asialo-G_{M1} will not cross-react with G_{M1} unless the binding is restricted to the region about the terminal βDGal unit. In fact, Marcus and co-workers[50] did observe that the IgG antibodies specific for asialo-G_{M1} did not cross-react with G_{M1}. The juxtaposition of the carboxyl group of G_{M1} next to a strongly hydrophobic topography is of major interest since the combination of the two surfaces to form an amphiphilic region may provide a clue as to how the important biological functions of gangliosides are expressed.

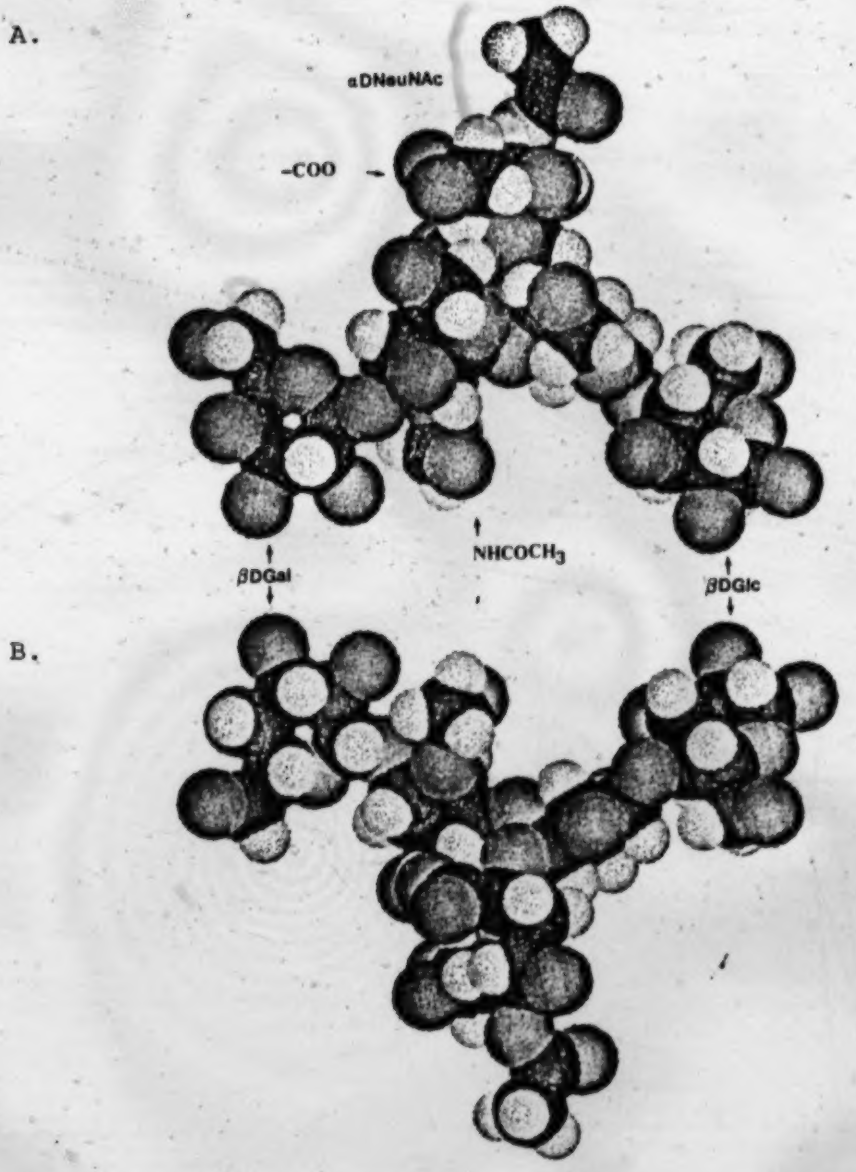

A.
αDNeuNAc
-COO
NHCOCH3
βDGal
βDGlc
B.

Fig.44 The computer drawn projections of the CPK model of the oligosaccharide portion of the ganglioside G_{M1} as derived by HSEA calculation: A. the view of the molecule along $H-2^{c}$ - $C-2^{c}$ bond; B. the view of the side of the molecule opposite to that of A.

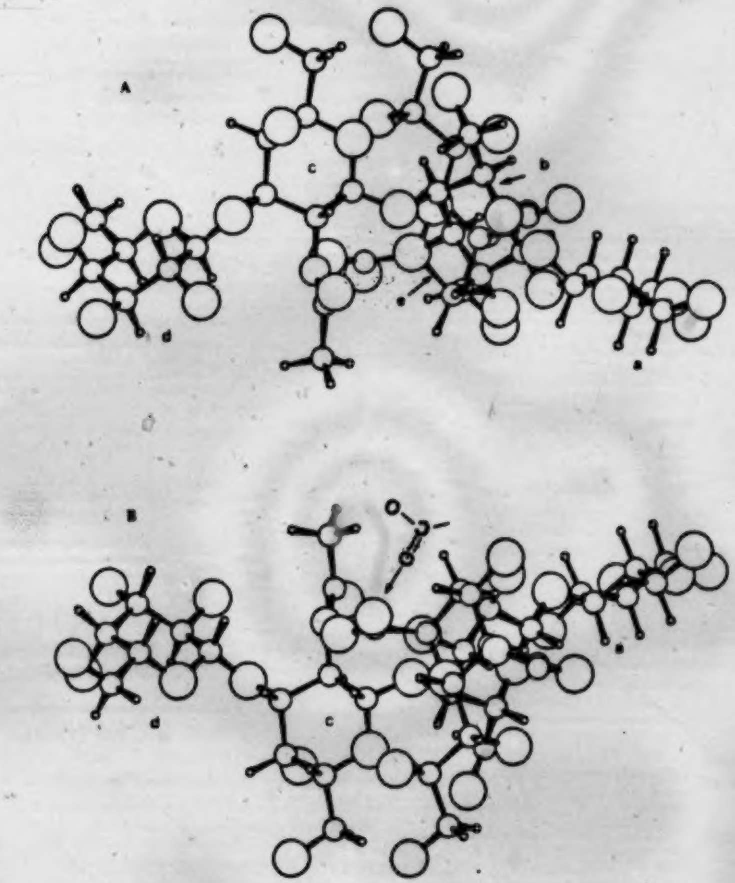


Fig.45 The computer drawn projections of the Drieding model of the oligosaccharide portion of the ganglioside G_{M1} as derived by HSEA calculation: A. the view of the molecule along $H-2^c$ - $C-2^c$ bond; B. the view of the side of the molecule opposite to that of A.

III. EXPERIMENTAL

All solvents and reagents were purified according to standard procedures.[120] All solid reactants for glycosylation were dried overnight over phosphorous pentoxide in a high vacuum prior to use. The molecular sieve (BDH 4 Å) was dried at 180°C for 24 h just prior to use. Solution transfers in these reactions were done under dry nitrogen using standard syringe techniques.[121] Removal of O-acetyl and O-benzoyl protecting groups was effected using a 0.5 M solution of sodium methoxide in dry methanol.

The solutions obtained in the course of solvent extractions were filtered through paper pre-wetted with the solvent and further dried over anhydrous magnesium sulphate before solvent removal with a rotary evaporator under the vacuum of a water aspirator and, unless otherwise indicated, at a bath temperature of 35°C or less.

Thin layer chromatograms (tlc) were performed on precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and visualized by charring after spraying with 5% sulphuric acid in ethanol. For column chromatography, silica gel H (type 60) (E. Merck, Darmstadt) and distilled solvents were used and the columns were loaded in the range 1:30 - 1:50.

Unless otherwise stated, proton magnetic resonance (^{1}H-nmr) spectra were recorded on a Brüker WH-200, 360 MHz or 400 MHz at 296°K. Carbon-13 nuclear magnetic resonance (^{13}C-nmr) spectra were recorded on a Brüker WH-200 (50.323 MHz) or WH-400 (100.614 MHz) at 305°K. ^{1}H- and ^{13}C-nmr chemical shifts are in parts per million (ppm) relative to internal 1% tetramethylsilane (TMS) in organic solvents. The ^{1}H-chemical shifts for compounds in deuterium oxide (D_2O) are expressed relative to HOD signal (4.81 ppm at 296°K) while the ^{13}C-chemical shifts are expressed using deuterium signal of the solvent as the reference. Whenever comparison of ^{13}C-chemical shifts among compounds is made, the same spectrometer and temperatures were used. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 589 nm in a 1 dm cell. The melting points are uncorrected.

8-Methoxycarbonyloctyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (16)

A solution of acetobromolactose[90] (13) (72.0 g, 103 mmol) in dichloromethane (400 mL) was added dropwise to a vigorously stirred suspension of anhydrous silver carbonate (56.5 g, 206 mmol), anhydrous calcium sulphate (40 g) and 8-methoxycarbonyloctanol[91] (36.2 g, 192.5 mmol) in dry dichloromethane (600 mL) over a period of 2 h. After stirring at room temperature for 16 h, the reaction mixture was filtered through a bed of diatomaceous earth (10 g) and evaporated to a syrup which was dissolved in a mixture of toluene-nitrobenzene (1:1, 800 mL) containing anhydrous mercuric bromide (1.0 g). After stirring for 24 h at 50°C, the reaction mixture was diluted with dichloromethane and washed with an aqueous potassium iodide solution (10%, 150 mL) and then finally with water (300 mL). Evaporation left a colorless syrup which was extracted with cyclohexane (4 x 100 mL) to remove the excess 8-methoxycarbonyloctanol (16.0 g). The yield of the crude syrup was 75.0 g.

A freshly prepared solution of sodium methoxide in anhydrous methanol (10 mL, 0.5 M) was added to a solution of the above syrup (75.0 g) in anhydrous methanol (500 mL) and stirred at room temperature for 6 h. A white suspension was obtained during this period which was cooled to 0°C, filtered and the residue was washed with ice-cold methanol (2 x 100 mL). The residue was crystallized from methanol (31.2 g, 59% yield) to form colorless crystals; m.p. 164-165°C. Lit. m.p., 159°C.

^{1}H-nmr (D_2O, HOD = 4.81 δ at 296°C)(Fig. 4) δ: 4.41 (t, 2H, H-1, H-1', $J_{1,2} = J_{1',2'} = 7.8$ Hz), 4.04 - 3.50 (m, 16 H, sugar ring protons, $O\text{-}C\underline{H}_3$, $O\text{-}C\underline{H}_2$ of aglycon), 3.25 (dd, 1H, H-2, $J_{2,1} = 7.8$ Hz, $J_{2,3} = 10$ Hz), 2.3 (t, 2H, $-C\underline{H}_2\text{-}COOCH_3$, J = 8 Hz), 1.7 and 1.4 (12H, hydrogens of aglycon.

^{13}C-nmr (D_2O)(Table) δ: 178.17 (-C=O), 103.22 (C-1'), 102.31 (C-1), 78.81 (C-4), 75.62 (C-5'), 75.02 (C-5), 74.76 (C-3), 73.14 (C-2), 72.85 (C-3'), 71.25 (C-2'), 70.97 (aglyconic carbon), 68.84 (C-4'), 61.27 (C-6'), 60.45 (C-6), 53.32 ($-COO\underline{C}H_3$), 33.97, 28.94, 28.65, 28.44, 28.39, 28.34, 25.17 and 24.51 (remaining carbons of aglycon).

8-Methoxycarbonyloctyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (15)

8-Methoxycarbonyloctyl β-lactoside (16) (1.0 g, 1.95 mmol) was dissolved in 10 mL of pyridine-acetic anhydride (1:1) and stirred at room temperature for 24 h. The reaction mixture was poured over ice (25 g) and extracted with dichloromethane (2 x 15 mL). The dichloromethane layer was washed with water, ice-cold HCl (1 N) and aqueous saturated sodium bicarbonate. Evaporation left a foam (1.40 g, 90% yield) which was homogeneous as examined by thin-layer chromatography (tlc). $[\alpha]_D^{25}$ -15.0° ($\underline{c}$ 1.33, $CHCl_3$); lit. $[\alpha]_D$ -11.2 ($\underline{c}$ 2, $CHCl_3$).

^{1}H-nmr ($CDCl_3$)(Fig.3) δ: 5.34 (dd, 1H, H-4', $J_{4',3'}$ = 3.5 Hz, $J_{4',5'}$ = 1 Hz), 5.20 (t, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9 Hz), 5.12 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 11 Hz), 4.94 (dd, 1H, H-3', $J_{3',4'}$ = 3.5 Hz, $J_{3',2'}$ = 11 Hz, 4.88 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 9 Hz), 4.52 (m, 3H, H-6a, H-1, H-1', $J_{1',2'}$ = 7.8 Hz, $J_{1,2}$ = 7.8 Hz), 4.19 - 4.07 (m, 3H, H-6'a, H-6'b, H-6b), 3.88 (dt, 1H, H-5', $J_{5',4'}$ = 1 Hz, $J_{5',6'}$ = 7 Hz), 3.85 - 3.76 (m, 2H, H-4, O-$C\underline{H}_2$ of aglycon), 3.7 (s, 3H, O-$C\underline{H}_3$), 3.62 (m, 1H, H-5), 3.46 (m, 1H, O-$C\underline{H}_2$ of aglycon), 2.3 (t, 2H, -$C\underline{H}_2$-$COOCH_3$, J = 8 Hz), 2.24 - 1.98 (7 x s, 21H, 7 x $C\underline{H}_3$ of acetyls), 1.58 and 1.38 (12H, hydrogens of aglycons).

^{13}C-nmr ($CDCl_3$) δ: 170.21, 170.00, 169.89, 169.43, 168.94 (8 x C=O), 100.98, 100.54 (C-1 and C-1'), 76.30 (C-4), 72.86, 72.59, 71.19, 70.96, 70.69, 70.01, 69.17, 66.65, 62.07, 60.81 (10 lines expected, 10 lines observed, the remaining carbons in sugar units and the aglyconic carbon), 51.31 - 20.38 (remaining carbons in aglycon and $\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{36}H_{54}O_{20}$: C 53.59, H 6.74; found: C 53.84, H 6.87.

8-Methoxycarbonyloctyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (17)

A suspension of compound 16 (24.0 g, 46.8 mmol) in dry acetonitrile (500 mL) was refluxed with α,α-dimethoxytoluene (14.25 g, 93.75 mmol) and *p*-toluenesulphonic acid monohydrate (50 mg) for 6 h. Examination of the clear reaction mixture by tlc (ethyl acetate-ethanol-water, 7:2:0.5) showed a single major compound. After neutralization of the solution with triethylamine (0.2 mL), the solvent was evaporated to dryness. The residue was extracted with *n*-hexane (3 x 50 mL) to remove the excess α,α-dimethoxytoluene in the residue. Crystallization from acetonitrile left colorless crystals (22.0 g, 78.2% yield); m.p. 186-188°C, $[\alpha]_D^{25}$ −32.1° (*c* 1.04, CH_3OH).

^{1}H-nmr (DMSO)(Fig. 5) δ: 7.50 - 7.32 (m, 5H, aromatic), 5.55 (s, 1H, benzylic), 5.28 - 4.50 (m, 5H, 5 x O-*H*), 4.37 and 4.16 (2 x d, 2H, two anomeric hydrogens, J = 7.8 Hz), 4.08 (d, 1H, H-4', $J_{4',3'}$ = 3.5 Hz), 4.08 and 4.02 (m, 2H, H-6'a, H-6'b), 3.48 - 3.0 (remaining sugar hydrogens), 2.28 (t, 2H, $-C\underline{H}_2-COOCH_3$, J = 8 Hz), 1.46 and 1.22 (12 H, remaining hydrogens in aglycon).

^{13}C-nmr (DMSO-d_6, TMS = 0) δ: 173.26 (*C*=O), 138.46, 128.54, 127.83, 126.16 (aromatic), 102.97, 102.59 (two anomeric carbons), 99.75 (benzylic carbon), 79.03 (C-4), 75.68, 74.83, 74.78, 73.24, 71.63, 69.82, 68.69, 68.43, 66.24, 60.31 (remaining carbons of the sugar units and the aglyconic carbon), 51.02, 33.22, 29.14, 28.61, 28.53, 25.34, 24.34 (remaining carbons in aglycon).

Anal. calcd. for $C_{29}H_{44}O_{13}$: C 57.96, H 7.38; found: C 57.69, H 7.21.

8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**18**)

A solution of compound **17** (21.0 g, 35 mmol) in pyridine-acetic anhydride (4:1, 125 mL) containing 4-*N*-*N*-dimethylaminopyridine (10 mg) was stirred at 60°C for 12 h. The reaction mixture was then poured over ice (100 g) and extracted with dichloromethane (3 x 200 mL). The dichloromethane layer was washed with water, ice-cold *N*-hydrochloric acid and finally with aqueous saturated sodium bicarbonate. Evaporation of the dried solution left a foam (28.3 g, 90% yield). An analytical sample was prepared by crystallization from ether-ethyl acetate-*n*-hexane; m.p. 160-162°C, $[\alpha]_D$ +32.0° (*c* 1.03, $CHCl_3$). A comparison of the ^{1}H-nmr spectrum of the crude product with that of the analytical sample showed that the former material was essentially pure and was therefore used directly in the next step.

^{1}H-nmr ($CDCl_3$)(Fig. 6) δ: 7.48 - 7.34 (m, 5H), 5.46 (s, 1H, benzylic), 5.24 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.5 Hz), 5.2 (t, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9 Hz), 4.90 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz , $J_{2,3}$ = 9 Hz), 4.86 (dd, 1H, H-3', $J_{3',4'}$ = 3.75 Hz, $J_{3',2'}$ = 10.5 Hz), 4.5 (dd, 1H, H-6a, $J_{6a,5}$ = 2.25 Hz, $J_{6a,6b}$ = 12 Hz), 4.45 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.42 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.32

(broad d, 1H, H-4', $J_{4',3'}$ = 3.75 Hz), 4.29 (broad d, 1H, H-6'a, $J_{6'a,6'b}$ = 12 Hz), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 5.25 Hz, $J_{6b,6a}$ = 12 Hz), 4.03 (broad d, 1H, H-6'b, $J_{6'b,6'a}$ = 12 Hz), 3.8 (m, 1H, O-C$\underline{H}_2$ of aglycon), 3.76 (t, 1H, H-4, $J_{4,3}$ = $J_{4,5}$ = 9 Hz), 3.66 (s, 3H, O-C$\underline{H}_3$), 3.60 (m, 1H, H-5), 3.45 (m, 2H, H-5' and -O-C$\underline{H}_2$- of aglycon), 2.3 (t, 2H, -C$\underline{H}_2$-COOCH_3, J = 8 Hz), 2.14 - 2.0 (5 x s, 15H, 5 x C$\underline{H}_3$ of acetyls), 1.6 and 1.28 (m, 12H, hydrogens in aglycon).

^{13}C-nmr (CDCl_3) δ: 174.12, 170.51, 170.34, 170.27, 169.51, 168.86 (6 x C=O), 137.75, 129.18, 128.25, 126.3, 101.36 and 101.22 (C-1 and C-1'), 100.88 (benzylic carbon), 77.50 (C-4), 73.33, 73.06, 72.77, 72.36, 72.22, 71.89, 70.06, 69.34, 68.54, 66.63, 62.30 (remaining carbons of sugar units and aglyconic carbon), 51.32 - 20.40 (9 lines, carbons in aglycon and $\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{39}H_{54}O_{18}$: C 57.74, H 6.71; found: C 57.5, H 6.66.

8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**19**)

Aqueous trifluoroacetic acid (90%, 10 mL) was added to a cold (0°C) solution of crude **18** (4.3 g) in dichloromethane (100 mL) and stirred at 0°C for 1 h. The reaction mixture was then washed with water, aqueous saturated sodium bicarbonate and finally with water. Evaporation left a dry residue which was purified on a column of silica gel (100 g) by flash chromatography using ethyl acetate-*n*-hexane-acetonitrile (2:2:1) as eluant. Evaporation of the second fraction left a foam (3.16 g, 82% yield). $[\alpha]_D^{25}$ -7.4 (*c* 1.05, $CHCl_3$).

^{1}H-nmr (DMSO-d_6)(Fig. 7) δ: 5.12 (d, 1H, 4'-OH, J = 4.5 Hz), 5.05 (t, 1H, 6'-OH, J = 9 Hz), 4.88 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10 Hz), 4.68 (dd, 1H, H-3', $J_{3',2'}$ = 10 Hz, $J_{3',4'}$ = 3.0 Hz), 4.62 (m, 3H, H-2, H-3, H-1), 4.47 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.25 (broad d, 1H, H-6a, $J_{6a,6b}$ = 12.0 Hz), 4.05 (dd, 1H, H-6b, $J_{6a,6b}$ = 12.0 Hz, $J_{6b,5}$ = 6.3 Hz), 3.90 (broad t, 1H, H-4', J = 3.0 Hz), 3.80 - 3.30 (m, remaining sugar hydrogens, O-C$\underline{H}_3$, O-CH_2 of aglycon), 2.30 (t, 2H, -C$\underline{H}_2$-$COOCH_3$, J = 8 Hz), 2.10 - 1.95 (15 H, 5 x C$\underline{H}_3$ of acetyls), 1.5 - 1.25 (12 H, hydrogens in aglycon).

^{13}C-nmr ($CDCl_3$) δ: 170.50, 170.08, 169.61, 169.41 (6 x C=O), 101.01, 100.60 (C-1' and C-1), 77.23 (C-4), 74.57, 73.50, 73.46, 72.70, 71.96, 70.09, 69.84, 68.17, 62.47, 62.41 (remaining carbons of sugar units and aglyconic carbon), 51.41 - 20.64 (carbons in aglycon and $C\underline{H}_3$ of acetyls).

8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**20**)

A solution of benzoyl chloride (0.640 g, 0.528 mL, 4.5 mmol) in dichloromethane (3 mL) was added to a cooled (-78°C) solution of compound **19** (2.96 g, 4.1 mmol) in dichloromethane (25 mL) containing anhydrous pyridine (0.363 mL, 4.5 mmol). All the starting diol (**19**) was consumed, as evidenced by tlc using ethyl acetate-*n*-hexane-acetonitrile (2:2:1), after stirring at -78°C for 3 h and then at -20°C for 6 h. The reaction mixture was washed with ice-cold water, ice-cold *N*-hydrochloric acid and finally with aqueous saturated sodium bicarbonate. Solvent removal left a solid foam which was purified by flash chromatography on a column of silica gel (60.0 g) using ethyl acetate-*n*-hexane-acetonitrile (2:2.5:0.5). Evaporation of the first main fraction left a colorless foam (2.8 g, 84% yield). An analytical sample was prepared by crystallization of the foam from acetone-*n*-hexane; m.p. 145-146°C, $[\alpha]_D^{25}$ +1.62° (*c* 0.986, $CHCl_3$).

A comparison of the ^1H-nmr spectrum of the foam (Fig. 8) with that of the analytical sample showed the former material to be essentially pure and therefore the foam was used in the glycosylation reactions.

^{1}H-nmr (DMSO-d_6)(Fig. 8) δ: 8.04, 7.70, 7.58 (m, 5H, benzoate), 5.18 (t, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9 Hz), 5.00 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10 Hz), 4.85 (dd, 1H, H-3', $J_{3',2'}$ = 10 Hz, $J_{3',4'}$ = 3.0 Hz), 4.68 (m, 3H, H-1, H-1', H-2), 4.48 (dd, 1H, H-6'a, $J_{6'a,5}$ = 5.25 Hz, $J_{6'a,6'b}$ = 11.5 Hz), 4.36 - 4.26 (m, 2H, H-6a, H-6'b), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 5.5 Hz, $J_{6b,6a}$ = 11.50 Hz), 4.03 (broad t, 1H, H-5', $J_{5',6'}$ = 6.0 Hz), 3.98 (broad d, 1H, H-4', $J_{4',3'}$ = 3.0 Hz), 3.82 (m, 1H, H-5), 3.75 - 3.64 (m, 5H, H-4 , $-O-C\underline{H}_3$, $-O-C\underline{H}_2$ of aglycon), 3.43 (m, 1H, $-O-C\underline{H}_2$ of aglycon), 2.28 (t, 2H, $-C\underline{H}_2-COOCH_3$, J = 8.0 Hz), 2.02 - 1.94 (15H, 5 x $C\underline{H}_3$ of acetyl), 1.5 and 1.24 (12H, hydrogens in aglycon).

^{13}C-nmr (CDCl$_3$) δ: 174.21, 170.39, 170.19, 169.44, 169.38, 166.40 (7 x $\overset{|}{\underset{|}{C}}=O$), 133.52, 129.74, 129.59, 128.70, 100.75, 100.17 (C-1 and C-1'), 76.41 (C-4), 73.57, 72.80, 72.67, 72.56, 71.75, 70.13 69.60, 66.95, 62.37, 62.18 (remaining carbons of sugar units and aglyconic carbon), 51.40 - 20.63 (10 lines, carbons in aglycon and $C\underline{H}_3$ of acetyls).

Anal. calcd. for $C_{39}H_{54}O_{19}$: C 56.65, H 6.58; found: C56.90, H 6.64.

Tri-*O*-acetyl-D-galactal (**23**)

Zinc dust (90.0 g, BDH) and aqueous copper sulphate solution (9.0 g $CuSO_4 \cdot 8H_2O$ in 33 mL water) were added to a vigorously stirred solution of sodium acetate trihydrate (163.7g, 1.2 mmol) in water-acetic acid (1:1, 1 L) cooled to 0°C. After 10 min, powdered acetobromogalactose (**22**)[101] (80.0 g, 194.6 mmol) was added over a period of 30 min and the stirring was continued for another 4 h at 0°C. The reaction mixture was filtered and diluted with water (750 mL). The filtrate was extracted with dichloromethane (3 x 500 mL) and the combined extract was washed with water and aqueous saturated sodium bicarbonate. Evaporation of the dried solvent left a colorless syrup (50.0 g, 94.5% yield), homogeneous (R_f = 0.42) as indicated by tlc using ethyl acetate-*n*-hexane (4:6). The ^{1}H-nmr spectrum of the product indicated that the purity of the material was greater than 90% (Fig. 9b). This was ascertained by comparison of the signal around 6.3 ppm due to H-1 with those of the acetyl signals around 2 ppm. The ^{1}H-nmr spectrum (Fig. 9b) was in accordance with the reported values.[103,104]

^{13}C-nmr ($CDCl_3$) δ: 170.45, 170.18, 170.06 (3 x $\underline{C}$=O), 145.44 (C-1), 98.92 (C-2), 72.89 (C-5), 63.95, 63.86 (C-3, C-4), 61.92 (C-6), 20.75, 20.71, 20.59 ($\underline{C}H_3$ of acetyls).

3,4,6-tri-O-Acetyl-2-azido-2-deoxy-α,β-D-galactopyranosyl nitrate (24) and N-acetyl-3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl amine (24a)

Compound 23 (42.2 g, 0.154 mmol) in acetonitrile (840 mL) was added to a mixture of sodium azide (15.0 g, 0.231 mol) and ceric ammonium nitrate (253.0 g, 0.462 mol) and cooled to -15°C. The resulting suspension was vigorously stirred with cooling until analysis by tlc (n-hexane-ethyl acetate (6:4), silica gel) of the supernatant liquid phase no longer showed the presence of compound 23. At this time, normally after an 8 to 10 h reaction period, cold diethyl ether (1000 mL) and water (1000 mL) were added. The organic layer was separated and washed with ice-cold water (3 x 500 mL) prior to drying. Evaporation of the solvent left a yellow syrup (45.0 g).

The ^{1}H-nmr spectrum was in accordance with that reported by Lemieux and Ratcliffe.[67]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (**25**)

Vilsmeier bromide[72] (10.0 g, 46 mmol) was added to the crude azido-nitration product **24** and **24a** (45.0 g) in dichloromethane (300 mL) and the solution was stirred at 0°C for 1 h. A solution of anhydrous lithium bromide (25.0 g, 287 mmol) in dry acetonitrile (350 mL) was then added and the stirring was continued for another 6 h. The reaction mixture was diluted with dry dichloromethane (300 mL) and washed with ice cold water (150 mL), saturated aqueous sodium bicarbonate (150 mL) and finally with distilled water (150 mL). The dried solution was evaporated to a yellow syrup (40.0 g). A portion of the syrup (10.0 g) was chromatographed on a column of silica gel (300 g) with ethyl acetate-*n*-hexane (3:7). The main fraction provided 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (7.0 g, 60% yield) which was crystallized from diethyl ether-hexane, m.p. 96-98°C.

The ^{1}H-nmr spectrum (Fig. 10c) of the recrystallized bromide was in accordance with that reported by Lemieux and Ratcliffe.[67]

A comparison of the ^{1}H-nmr spectrum of the sample before column purification with that of the analytical sample (Fig. 10c) showed that the former product was about 75% pure.

The crude bromide was employed in the next step, the glycosylation reaction, without purification.

^{13}C-nmr ($CDCl_3$) δ: 170.22, 169.70, 169.43 (3 x $\overset{|}{\underset{|}{\mathrm{C}}}{=}\mathrm{O}$), 89.07 (C-1), 71.53 (C-5), 69.84, 66.72 (C-4 and C-3), 60.78 (C-6), 58.75 (C-2).

Allyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (**26**)

A solution of the crude bromide **25** (30.0 g, 7.61 mmol) in dichloromethane (125 mL) was added in drops to a vigorously stirred suspension of anhydrous silver carbonate (20.0 g, 7.25 mmol) and anhydrous calcium sulphate (25.0 g) in allyl alcohol (200 mL). After stirring at room temperature for 16 h, the reaction mixture was filtered through a bed of diatomaceous earth (10.0 g) and the filtrate was evaporated to dryness. Purification by flash column chromatography on silica gel (600 g) using ethyl acetate-*n*-hexane (3:7) and the evaporation of the first main fraction resulted in a pale yellow syrup (15.97 g, 29% based on acetobromogalactose) which was homogeneous as evidenced by tlc. $[\alpha]_D^{25}$ -12.4° (*c* 1.5, $CHCl_3$).

^{1}H-nmr ($CDCl_3$) (Fig. 11) : 5.96 (m, 1H, $-O-CH_2-C\underline{H}=CH_2$), 5.42 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 5.40 (broad d, 1H, H-4, $J_{4,3}$ = 3 Hz), 5.32 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 4.84 (dd, 1H, H-3, $J_{3,4}$ = 3 Hz, $J_{3,2}$ = 10.5 Hz), 4.46 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.45 and 4.24 (m, 2H, $-O-C\underline{H}_2-CH=CH_2$), 4.3 - 4.2 (m, 2H, H-6a, H-6b), 3.88 (broad t, 1H, H-5, $J_{5,6}$ = 6.75 Hz), 3.75 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz), 2.08 - 2.04 (3 x s, 9H, $C\underline{H}_3$ of acetyls.

^{13}C-nmr ($CDCl_3$) δ: 170.30, 170.04, 169.77 (3 x $\overset{|}{\underset{|}{C}}{=}O$), 133.14 ($-O-CH_2-\underline{C}H{=}CH_2$), 118.25 ($-O-CH_2-CH{=}CH_2$), 101.17 (C-1), 71.14 (C-5), 70.73, 70.64 (C-3 and C-4), 66.44 ($-O-\underline{C}H_2-CH{=}CH_2$), 61.29 (C-2), 60.93 (C-6), 20.59 (3 x $\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{15}H_{21}N_3O_{18}$: C 48.49, H 5.70, N 11.32; found: C 47.98, H 5.66, N 10.65.

Allyl 2-azido-2-deoxy-β-D-galactopyranoside (**27**)

A freshly prepared solution of sodium methoxide in methanol (5 mL, 0.5 mol) was added to a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (**26**) (18.0 g, 48.5 mmol) in methanol (150 mL). After stirring at room temperature for 90 min, the solution was neutralized with IRA-120 H^+ resin. The yellow colored solution was decolorized with charcoal (5.0 g) and evaporated. The colorless solid was crystallized from ethyl acetate-*n*-hexane to obtain 11.0 g (92.3%) of the recrystallized product; m.p. 117-118°C, $[\alpha]_D^{25}$ +13.9° (*c* 0.98, CH_3OH).

^{1}H-nmr (CD_3OD, HOD = 4.81) (Fig. 12) δ: 6.04 - 5.90 (m, 1H, $-O-CH_2-C\underline{H}=CH_2$), 5.35 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 5.18 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 4.34 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 3.78 (dd, 1H, H-4, $J_{4,5}$ = 1.5 Hz, $J_{4,3}$ = 3.5 Hz), 3.74 (m, 2H, H-6a, H-6b), 3.48 (m, 2H, H-2, H-5), 3.42 (dd, 1H, H-3, $J_{3,4}$ = 3.5 Hz, $J_{3,2}$ = 10.5 Hz).

^{13}C-nmr (DMSO-d_6) δ: 134.37 ($-O-CH_2-\underline{C}H=CH_2$), 116.43 ($-O-CH_2-CH=\underline{C}H_2$), 100.41 (C-1), 75.20 (C-5), 71.55 (C-3), 68.80 (C-4), 67.55 ($-O-\underline{C}H_2-CH=CH_2$), 64.22 (C-2), 60.26 (C-6).

Anal. calcd. for $C_9H_{15}N_3O_5$: C 44.06, H 6.16, N 17.14; found: C 43.59, H 6.21, N 17.00.

Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (29)

A slow stream of hydrogen sulphide was bubbled through an ice-cold solution of the azide (27) (14.0 g, 57.14 mmol) in pyridine (150 mL) and triethylamine (45 mL) for 4 h. The solution was then stirred at room temperature for another 6 h and then evaporated to dryness. The residue (28) was extracted with a mixture of methanol-water (1:1), filtered through a bed of diatomaceous earth and evaporated.

Phthalic anhydride (4.86 g, 32.83 mmol) was added to a solution of the above residue in pyridine (50 mL) and heated to 60°C for 30 min. Triethylamine (5.9 g, 57.28 mmol) and more phthalic anhydride (4.86 g, 32.83 mmol) were added and the heating was continued for 2 h. Methanol (50 mL) was added and the solution was evaporated to a dry residue which was taken up in a mixture of pyridine-acetic anhydride (2:1, 60 mL). 4-*N*,*N*-dimethylaminopyridine (10 mg) was added and the solution was stirred at 60°C for 24 h. The reaction mixture was then poured over crushed ice (100 g) and the product was extracted with dichloromethane (2 x 150 mL). The dichloromethane layer was washed with water, ice-cold hydrochloric acid (1N), and finally with aqueous saturated sodium bicarbonate. After drying, the solution was evaporated to a foamy residue which was purified by flash chromatography on a column of silica gel (600 g) using ethyl acetate-hexane-acetonitrile (2:2.5:0.5). Evaporation of the first main fraction left a colorless foam (19.0 g, 70% yield) homogeneous on tlc. $[\alpha]_D^{25}$ -10.7° ($\underline{c}$ 1.0, $CHCl_3$).

^1H-nmr ($CDCl_3$)(Fig. 13) : 7.80 - 7.72 (m, 4H, phthalimido), 5.8 (dd, 1H, H-3, $J_{3,2}$ = 11 Hz, $J_{3,4}$ = 3.5 Hz), 5.78 - 5.66 (m, 1H, $\mathrm{O{-}CH_2{-}C\underline{H}{=}CH_2}$), 5.50 (broad d, 1H, H-4, $J_{4,3}$ = 3.5 Hz), 5.35 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 5.14 (m, 1H, $\mathrm{O{-}CH_2{-}CH{=}C\underline{H}_2}$), 5.08 (m, 1H, $\mathrm{{-}O{-}CH_2{-}CH{=}C\underline{H}_2}$), 4.60 (d, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.0 Hz), 4.34 - 4.04 (m, 5H, H-6a, H-6b, H-5, $\mathrm{{-}O{-}C\underline{H}_2{-}CH{=}CH_2}$), 2.22, 2.1, 1.86 (3 x s, $\mathrm{C\underline{H}_3}$ of acetyls).

^{13}C-nmr ($CDCl_3$) δ: 170.39, 169.79 (C=O), 134.27, 131.53, 123.78, 123.58 (aromatic carbons), 133.32 ($\mathrm{O{-}CH_2{-}\underline{C}H{=}CH_2}$), 117.84 ($\mathrm{O{-}CH_2{-}CH{=}\underline{C}H_2}$), 97.56 (C-1), 70.87, 70.17, 68.15 (C-5, C-4, C-3), 66.83 ($\mathrm{O{-}\underline{C}H_2{-}CH{=}CH_2}$), 61.50 (C-6), 51.46 (C-2), 20.70, 20.50, 20.36 (3 x $\mathrm{\underline{C}H_3{-}COO{-}}$).

Anal. calcd. for $C_{23}H_{25}NO_{10}$: C 58.10, H 5.30, N 2.94; found: C 57.82, H 5.30, N 2.90.

3,4,6-tri-O-Acetyl-2-deoxy-2-phthalimido-D-galactopyranose (31)

Tris-triphenylphosphine rhodium (III) chloride (300 mg, 0.32 mmol) and 1,4-diazabicyclooctane (100 mg, 1.1 mmol) were added to a solution of compound 29 (2.5 g, 5.26 mmol) in ethanol - benzene - water (7:3:1, 300 mL) and refluxed for 36 h. The solvents were then evaporated to dryness and the residue was dissolved in aqueous acetone (90%, 50 mL) containing mercuric chloride (5 g) and yellow mercuric oxide (100 mg). After stirring at room temperature for 60 min, the reaction mixture was diluted with water (50 mL) and the product was extracted with chloroform (2 x 75 mL). The chloroform layer was washed with aqueous potassium iodide (10%, 2 x 50 mL) and then with water. Evaporation left a black residue which was purified by flash chromatography on a column of silica gel (100 g) using ethyl acetate-hexane-acetonitrile (2:2.5:0.5). Evaporation of the second main fraction left a foam (1.88 g, 78%) which was a mixture of α and β anomers of 31, as evidenced by the ^{1}H-nmr spectrum (Fig. 15).

^{1}H-nmr ($CDCl_3$) (Fig. 15) δ: 6.42 (dd, H-3α, $J_{3\alpha,2\alpha}$ = 11 Hz, $J_{3\alpha,4\alpha}$ = 3.0 Hz), 5.84 (dd, H-3β, $J_{3\beta,2\beta}$ = 11 Hz, $J_{3\beta,4\beta}$ = 3.5 Hz), 5.46 - 5.36 (m, H-4, H-1α, H-1β), 4.84 - 3.06 (H-2α, H-2β, H-5, H-6a, H-6b), 1.84 - 2.16 (m, $C\underline{H}_3$ of acetyls).

3,4,6-tri-O-Acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl bromide (**32**)

Vislmeier bromide (1.31 g, 6.05 mmol) was added to an ice-cold solution of compound **31** (1.88 g, 4.32 mmol) in dichloromethane (25 mL) and stirred. sym-Collidine (0.802 mL, 6.05 mmol) was added in drops to the reaction mixture. After stirring for 2 h at 0°C and 12 h at room temperature, the reaction mixture was washed with ice-cold water (15 mL), ice-cold hydrochloric acid (1 N, 10 mL) and finally with water. Evaporation of the solvent left a residue (1.88 g, 84% yield) containing α and β anomers of **32**. The pure β-bromide was crystallized out from the mixture using ethylacetate-n-hexane; m.p. 136-137°C, $[\alpha]_D^{25}$ +29.2° (c 0.98, $CHCl_3$).

^{1}H-nmr ($CDCl_3$) (Fig. 16) δ: 7.86 - 7.70 (m, 4H, phthalimido), 6.36 (d, 1H, H-1, $J_{1,2}$ = 10 Hz), 5.76 (dd, 1H, H-3, $J_{3,2}$ = 11.0 Hz, $J_{3,4}$ = 3.50 Hz), 5.52 (broad d, 1H, H-4, $J_{4,3}$ = 3.50 Hz), 4.8 (dd, 1H, H-2, $J_{2,3}$ = 11 Hz, $J_{2,1}$ = 10 Hz), 4.17 (m, 3H, H-5, H-6a, H-6b), 2.28, 2.08, 1.84 (3 x s, $C\underline{H}_3$ of acetyls).

^{13}C-nmr ($CDCl_3$) δ: 170.27, 170.09, 169.45 (3 x C=O), 134.53, 131.30, 123.83 (aromatic), 78.08 (C-1), 75.88, 67.88, 66.66 (C-5, C-3, C-2), 61.38 (C-6), 54.61 (C-2), 20.64, 20.38 ($\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{20}H_{20}BrNO_9$: C 48.20, H 4.04, N 2.82; found: C 48.01, H 4.10, N 2.81.

8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,di-*O*-acetyl-6-*O*-benzoyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (33)

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-D-galactopyranosyl bromide (32)(225 mg, 0.45 mmol) in nitromethane (5 mL) was added to a solution of the alcohol 20 (340 mg, 0.41 mmol), *sym*-collidine (54.5 µL, 0.411 mmol) and silver triflate (115 mg, 0.45 mmol) in dry nitromethane (5 mL) cooled to -25°C. The mixture was stirred at -25°C for 6 h and then at room temperature for 12 h. The solids that were deposited after the addition of dichloromethane (20 mL) were removed by filtration and the filtrate was washed with sodium thiosulphate solution, cold water, dilute aqueous HCl and saturated sodium bicarbonate solution. Solvent removal after drying left a foam which was purified by preparative high pressure liquid chromatography on a column of silica gel using ethyl acetate-*n*-hexane-acetonitrile (2:2.5:0.5) which gave a colorless foam (330 mg, 64.5% yield), $[\alpha]_D^{25}$ -19.9° (*c*, 2.7, $CHCl_3$).

^{1}H-nmr ($CDCl_3$) (Fig. 17) δ: 8.0 - 7.5 (broad m, 4H, phthalimido), 5.92 (dd, 1H, H-3", $J_{3",4"}$ = 3.5 Hz, $J_{3",2"}$ = 11 Hz), 5.44 (broad d, 1H, H-4", $J_{4",3"}$ = 3.5 Hz), 5.32 (d, 1H, H-1", $J_{1",2"}$ = 7.8 Hz), 5.15 (t, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 10 Hz), 4.84 (m, 2H, H-2, H-3', $J_{2,3}$ = 10 Hz, $J_{2,1}$ = 7.8 Hz, $J_{3',2'}$ = 10 Hz, $J_{3',4'}$ = 3.0 Hz), 4.70 (broad d, 1H, H-6'a, $J_{6'a,6'b}$ = 11 Hz, $J_{6'a,5'}$ = 7.0 Hz), 4.52 (m, 2H, H-2", H-2'), 4.40 (m, 3H, H-6a, H-1, H-6'b), 4.28 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.18 (broad d, 1H, H-4', $J_{4',3'}$ = 3.0 Hz), 4.12 - 3.94 (m, 4H, H-5", H-6"a, H-6"b), 3.8 (m, 2H, H-5', O-C$\underline{H}_2$ of aglycon), 3.70 (m, 4H, O-C$\underline{H}_3$, H-4), 3.51 (m, H-1, H-5), 3.4 (m, 1H, O-C$\underline{H}_2$ of aglycon), 2.3 (t, 2H, -C$\underline{H}_2$-COOCH_3, J = 8.0 Hz), 2.2 - 1.74 (8 x s, 24H, acetyl CH_3), 1.6 and 1.20 (12H, aglyconic hydrogens).

^{13}C-nmr (CDCl_3) δ: 174.21 ($\underline{C}$OOCH_3), 170.33, 170.30, 170.27, 170.07, 169.72, 169.18, 168.59, 167.98, 167.16, 166.26 (10 x $\overset{|}{\underset{|}{\mathrm{C}}}$=O), 134.28, 133.97, 133.44, 131.71, 129.97, 128.77, 124,58, 123.07 (phthalimido, benzoate carbons), 100.92, 100.41, 98.46 (C-1, C-1', C-1"), 77.28 (C-4), 75.42, 72.89, 72.48, 72.25, 72.15, 71.83, 71.04, 70.04, 69.57, 57.49, 66.67, 62.13, 62.12, 61.41, 51.38 (remaining carbons of sugar units and the -O-$\underline{C}H_2$- of aglycon), 51.28 - 20.29 (12 lines, remaining carbons in aglycon, $\underline{C}H_3$ of acetyls).

8-Methoxycarbonyloctyl 4-*O*-[4-*O*-(2-deoxy-2-phthalimido-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**34**)

A freshly prepared solution of sodium methoxide in methanol (0.5 mL, 0.5 M) was added to a solution of compound **33** (364 mg, 0.289 mmol) in anhydrous methanol (20 mL) and stirred under nitrogen atmosphere for 5 h. The reaction mixture was neutralized with dry IRA-120 H^+ resin and evaporated to a dry residue, which was homogeneous on tlc (ethyl acetate-ethanol-water, 7:2:1), to yield 220 mg (97%).

^{1}H-nmr (CD_3OD, HOD = 4.81δ) δ: 8.0 - 7.7 (m, 4H, phthalimido), 5.15 (d, 1H, H-1", $J_{1",2"}$ = 8.0 Hz), 4.59 (dd, 1H, H-3", $J_{3",2"}$ = 11.0 Hz, $J_{3",4"}$ = 3.50 Hz), 4.33 (dd, 1H, H-2", $J_{2",1"}$ = 8.0 Hz, $J_{2',3'}$ = 11.0 Hz), 4.23 - 4.15 (2 x d, 2H, H-1, H-1', $J_{1',2'} = J_{1,2}$ = 7.8 Hz), 3.9 - 3.25 (m, remaining sugars and aglycones except H-2 and H-2'), 3.18 (t, 1H, H-2, $J_{2,3} = J_{1,2}$ = 9.0 Hz), 3.07 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 9.5 Hz), 2.27 (t, 2H, $C\underline{H}_2COOCH_3$, J = 8.0 Hz), 1.55 and 1.29 (12H, hydrogens in aglycon).

8-Methoxycarbonyloctyl 4-*O*-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**8**) — *asialo*-G_{M2} hapten

Hydrazine (95%, 100 μL) was added to a solution of compound **34** (219 mg, 0.280 mmol) in methanol (20 mL) and refluxed for 2 h. The reaction mixture was then evaporated to a dry residue which was taken up in 11 mL of methanol-acetic anhydride (10:1) and stirred at room temperature for 3 h. After evaporation to a dry residue, the crude product was filtered through a Bio-gel P-2 column using ethanol-water (9:1). The main fractions on lyphophilization left *asialo*-G_{M2} hapten (**8**) as a colorless material (155 mg, 79.89% yield). $[\alpha]_D^{25}$ -0.1 (*c*, 0.3).

^{1}H-nmr (D_2O, HOD = 4.81) (Fig. 19 and Table 4) : 4.58 (d, 1H, H-1", $J_{1'',2''}$ = 9 Hz), 4.43, 4.39 (2 x d), 2H, H-1, H-1', $J_{1',2'}$ = $J_{1,2}$ = 8.0 Hz), 4.04 (broad d, 1H, H-4', $J_{4',3'}$ = 2.2 Hz), 4.0 - 3.35 (20H, remaining 15 ring hydrogens except H-2' and H-2; O-CH_3, O-CH_2 of aglycon), 3.36 (dd, 1H, H-2', $J_{2',1'}$ = 7.7 Hz, $J_{2',3'}$ = 9.9 Hz), 3.24 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.0 Hz), 2.4 (t, 2H, -$C\underline{H}_2$-$COOCH_3$), 2.05 (s, 3H, CH_3 of N-acetyl), 1.6 and 1.30 (12H, aglyconic hydrogens).

^{13}C-nmr (D_2O) (Fig. 20 and Table 5) δ: 178.09 ($-\underline{C}OOCH_3$), 175.27 ($-NH\underline{C}OCH_3$), 103.34 (C-1'), 102.94 (C-1"), 102.37 (C-1), 79.04 (C-4), 76.48 (C-4'), 75.21 (C-5"), 75.10 (C-5), 74.78 (C-5'), 74.76 (C-3), 73.16 (C-2), 72.90 (C-3'), 71.45 (C-3", C-2'), 71.02 (aglyconic carbon), 68.21 (C-4"), 61.41 (C-6"'), 61.03 (C-6'), 60.53 (C-6), 53.70 ($COO\underline{C}H_3$), 52.37 - 22.74 (8 lines remaining carbon in aglycone and $\underline{C}H_3$-CO-NH-).

Allyl 2-azido-2-deoxy-4,6-*O*-benzylidene-β-D-galactopyranoside (**35**)

A solution of compound **27** (11.0 g, 44.8 mmol) in dry acetonitrile (200 mL) containing α,α-dimethoxytoluene (11.0 g, 72.3 mmol) and *p*-toluenesulphonic acid (150 mg, 0.87 mmol) was stirred at room temperature for 16 h. The solution was then neutralized with triethylamine and evaporated to dryness. A solution of the residue in dichloromethane (150 mL) was washed with water (50 mL), dried and evaporated to leave a pale yellow solid which crystallized from toluene (11.8 g, 79% yield); m.p. 129-131°C, $[\alpha]_D^{25}$ -15.3° (*c* 1.03, $CHCl_3$).

^{1}H-nmr ($CDCl_3$) (Fig. 21) δ: 7.48 - 7.34 (m, 5H, aromatic), 6.02 - 5.9 (m, 1H, $O\text{-}CH_2\text{-}C\underline{H}\text{=}CH_2$), 5.56 (s, 1H, benzylic) 5.34 (m, 1H, $O\text{-}CH_2\text{-}CH\text{=}C\underline{H}_2$), 5.22 (m, 1H, $O\text{-}CH_2\text{-}CH\text{=}C\underline{H}_2$), 4.44 and 4.14 (m, 3H, H-4, $O\text{-}C\underline{H}_2\text{-}CH\text{-}CH_2$), 4.31, (m, 2H, H-1, H-6a), 4.04 (bd, J = 12.2 Hz, 1H, H-6b), 3.64 (dd, J = 8,10 Hz, 1H, H-2), 3.56 (ddd, J = 10.0 Hz, 8, 3.5 Hz, 1H, H-3), 3.41 (broad s, 1H, H-5), 2.59 (d, J = 8.0 Hz, 1H, -O-*H*).

^{13}C-nmr ($CDCl_3$) δ: 133.58 ($O\text{-}CH_2\text{-}CH_2\text{-}\underline{C}H\text{=}CH_2$), 129 - 126.0 (3 lines, aromatic carbons), 117.76 ($O\text{-}CH_2\text{-}CH\text{=}\underline{C}H_2$), 101.46, 100.93 (C-1, benzylic carbon), 74.61 (C-4), 71.55 (C-5), 70.23 (C-3), 69.02 (C-6), 66.56 (O-$\underline{C}H_2$-CH=CH_2), 64.10 (C-2).

Anal. calcd. for $C_{16}H_{19}N_3O_5$: C 57.62, H 5.74, N 12.61; found: C 57.48, H 5.85, N 12.58.

Allyl-2-azido-2-deoxy-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (36)

A solution of acetobromogalactose[101] (7.36 g, 19.7 mmol) in dry nitromethane (30 mL) was added to a cooled (-25°C) solution of the alcohol 35 (5.97 g, 17.9 mmol), silver trifluoromethanesulphonate (5.067 g, 19.7 mmol) and sym-collidine (2.37 mL, 17.9 mmol) in dry nitromethane (100 mL). After stirring at -25°C for 8 h, the reaction mixture was diluted with dichloromethane (200 mL) and filtered through a bed of diatomaceous earth. The filtrate was washed with water, aqueous saturated sodium bicarbonate and finally with water. Evaporation of the solvent left a residue which was purified by flash chromatography on a column of silica gel (300 g) using ethyl acetate-n-hexane-acetonitrile (2:2.5:0.5). The first main fraction on evaporation left a foam (8.3 g, 70% yield) homogeneous on tlc.

An analytical sample was prepared by crystallization of the foam from methanol; m.p. 141-144°C, $[\alpha]_D^{25}$ +13.7° (c 0.96, $CHCl_3$).

A comparison of the ^1H-nmr spectrum of the analytical sample with that of the foam indicated the latter to be essentially pure and hence the foam was used in subsequent steps.

^{1}H-nmr ($CDCl_3$) (Fig. 22) δ: 7.52 - 7.35 (m, 5H, aromatic), 6.0 - 5.8 (m, 1H, $O\text{-}CH_2\text{-}C\underline{H}{=}CH_2$), 5.54 (s, 1H, benzylic), 5.39 (dd, 1H, H-4', $J_{4',3'}$ = 3.0 Hz, $J_{4',5'}$ = 1.5 Hz), 5.32 (m, 1H, $\text{-}O\text{-}CH_2\text{-}CH{=}C\underline{H}_2$), 5.22 (m, 1H, $\text{-}O\text{-}CH_2\text{-}CH{=}C\underline{H}_2$), 5.26 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.0 Hz), 5.02 (dd, 1H, H-3', $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.0 Hz), 4.8 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.4 (m, 1H, $O\text{-}C\underline{H}_2\text{-}CH{=}CH_2$), 4.33 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.31 (dd, 1H, H-6a, $J_{6a,5}$ = 1.5 Hz, $J_{6a,6b}$ = 12.0 Hz), 4.21 (broad d, 1H, H-4, $J_{4,3}$ = 3.5 Hz), 4.19 - 4.09 (m, 3H, H-6'a, H-6'b, $O\text{-}C\underline{H}_2\text{-}CH{=}CH_2$), 4.04 (dd, 1H, H-6b, $J_{6b,5}$ = 1.5 Hz, $J_{6b,6a}$ = 12.0 Hz), 3.89 (broad t, 1H, H-5', $J_{5',6'}$ = 6.5 Hz), 3.82 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz), 3.46 (dd, 1H, H-3, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz), 3.36 (broad s, H-5), 2.16 - 1.98 (4 x s, 12H, 4 x $C\underline{H}_3$ of acetyls).

^{13}C-nmr ($CDCl_3$) δ: 170.27, 170.10, 169.28 (4 lines expected, 3 lines observed, 4 x $\text{-}O\text{-}\underline{C}OCH_3$), 137.86, 128.89, 126.31 (aromatic carbons), 133.7 ($\text{-}O\text{-}CH_2\text{-}\underline{C}H{=}CH_2$), 117.61 ($O\text{-}CH_2\text{-}CH{=}\underline{C}H_2$), 102.32 (C-1'), 101.23 (C-1), 100.82 (benzylic), 78.63 (C-3), 75.11 (C-4), 71.03, 70.10, 69.01, 68.95, 67.17, 66.70, 62.44, 61.47 (9 lines expected, 8 lines observed, the remaining carbons of sugar units and the aglyconic carbon), 20.65, 20.52 ($\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{30}H_{37}N_3O_{14}$: C 54.27, H 5.62, N 6.33; found: C 54.37, H 5.72, N 6.11.

Allyl 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (37)

Aqueous trifluoroacetic acid (90%, 10 mL) was added to a solution of compound 36 (5.0 g) in dichloromethane (100 mL) kept at 0°C. After stirring for 30 min, the solution was washed with ice-cold water (50 mL), aqueous saturated sodium bicarbonate and finally with water. Solvent removal left a syrup which was dissolved in dry dichloromethane (100 mL) containing pyridine (5 mL) and acetic anhydride (5 mL). 4-*N*,*N*-Dimethylaminopyridine (10 mg) was added to the above mixture and stirred at room temperature for 12 h. The reaction mixture was then washed with ice-cold hydrochloric acid (1 N), saturated aqueous sodium bicarbonate and finally with water. The dried solution was evaporated to a syrup and passed through a short silica gel column to remove the benzaldehyde impurity formed at the initial step. Evaporation of the second main fraction left a solid (3.9 g, 80% yield) which was homogeneous on tlc; m.p. 111-112°C, $[\alpha]_D^{25}$ +10.0 ($\underline{c}$ 1.89, $CHCl_3$).

^{1}H-nmr ($CDCl_3$)(Fig. 26) δ: 6.0 - 5.88 (m, 1H, $O\text{-}CH_2\text{-}\underline{CH}{=}CH_2$), 5.4 - 5.22 (m, 4H, H-4, H-4', $O\text{-}CH_2\text{-}CH{=}\underline{CH_2}$), 5.15 (dd, 1H, H-2', $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 10.5 Hz), 5.0 (dd, 1H, H-3', $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz), 4.71 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.42 (m, 1H, $O\text{-}C\underline{H_2}\text{-}CH{=}CH_2$), 4.33 (d, 1H, H-1,

$J_{1,2}$ = 8.0 Hz), 4.22 - 4.04 (m, 5H, H-6a, H-6b, H-6'a, H-6'b, O-$C\underline{H}_2$-CH=CH_2), 3.90 (broad t, 1H, H-5', $J_{5',6'}$ = 7.0 Hz), 3.75 (broad t, 1H, H-5, $J_{5,6}$ = 7.0 Hz), 3.64 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz), 3.54 (dd, 1H, H-3, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 3.5 Hz), 2.2 - 2.04 (6 x s, 18H, $C\underline{H}_3$-COO-).

^{13}C-nmr ($CDCl_3$) δ: 170.47, 170.34, 170.25, 170.07, 169.75 159.29 (6 x $\overset{|}{\underset{|}{C}}$=O), 133.22 (-O-$CH_2$-$\underline{C}$H=$CH_2$), 118.09 (O-$CH_2$-CH=$\underline{\underline{C}}H_2$), 101.47, 101.00 (anomeric carbons C-1 and C-1'), 77.26 (C-3), 71.42, 70.84, 70.66, 70.47, 68.85, 68.10 (C-5, C-5', C-3', C-2', C-4', C-4), 66.83 (O-$\underline{C}H_2$-CH=CH_2), 63.34 (C-2), 62.29, 61.02 (C-6, C-6'), 20.70, 20.65, 20.63, 20.53 (6 x $\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{27}H_{37}N_3O_{16}$: C 49.16, H 5.65, N 6.37; found: C 49.27, H 5.68, N 6.27.

Allyl 2-azido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranoside (**38**)

A freshly prepared solution of sodium methoxide in methanol (2.5 mL, 0.5 M) was added to a solution of **37** (4.0 g) in anhydrous methanol (75 mL) and stirred at room temperature for 6 h. After neutralization with IRA-120 H^+ resin, the solution was evaporated to a dry residue (2.3 g, 93.5% yield) which was homogeneous on tlc (ethyl acetate-ethanol-water, 7:2:0.5). An analytical sample was prepared by crystallization of a small quantity of the residue from ethanol-ether; m.p. 168-170°C (dec.), $[\alpha]_D^{25}$ +12.0 (*c* 0.64, H_2O). A comparison of the ^{1}H-nmr spectrum (Fig. 26) of the analytical sample with that of the residue showed the latter to be essentially pure and the residue was used in subsequent steps.

^{1}H-nmr (D_2O, HOD = 4.81) (Fig. 27) : 5.98 (m, 1H, $-O-CH_2-C\underline{H}=CH_2$), 5.34 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 5.25 (m, 1H, $-O-CH_2-CH=C\underline{H}_2$), 4.54 and 4.48 (2 x d, 2H, H-1', H-1, $J_{1',2'} = J_{1,2} =$ 7.8 Hz), 4.1 (dd, 1H, H-4, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.5 Hz), 3.85 (broad d, 1H, H-4', $J_{4',3'}$ = 3.5 Hz), 3.78 - 3.44 (m, 10H, remaining hydrogens of sugar units).

^{13}C-nmr (D_2O) δ: 133.33 ($-O-CH_2-\underline{C}H=CH_2$), 118.98 ($-O-CH_2-CH=\underline{C}H_2$), 104.82 (C-1'), 100.82 (C-1), 80.29 (C-3), 75.20 (C-5'), 75.00 (C-5), 72.69 (C-3'), 70.89 (C-2'), 68.73 (C-4, C-4'), 68.07 ($-O-\underline{C}H_2-CH=CH_2$), 62.77 (C-2), 61.15, 60.96 (C-6' and C-6).

Allyl 2-amino-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranoside (**39**)

A slow stream of hydrogen sulphide was bubbled through an ice-cold solution of the crude **38** (2.3 g) in pyridine (30 mL) and triethylamine (10 mL) for 4 h. The reaction mixture was then stirred at room temperature for another 12 h. The solution was evaporated to dryness and the residue was extracted with water. The extract was filtered through a bed of diatomaceous earth (5.0 g) and evaporated. The residue (2.3 g) appeared to be the desired amine as assessed by tlc as well as ^{1}H- and ^{13}C-nmr.

^{1}H-nmr (D_2O, HOD = 4.81)(Fig. 28) δ: 6.0 - 5.88 (m, 1H, O-CH$_2$-C$\underline{\text{H}}$=CH$_2$), 5.35 (m, 1H, O-CH$_2$-CH=C$\underline{\text{H}}_2$), 5.25 (m, 1H, O-CH$_2$-CH=C$\underline{\text{H}}_2$), 4.50 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz, 4.36 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.36 (m, 1H, O-C$\underline{\text{H}}_2$-CH=CH$_2$), 4.18 (m, 1H, O-C$\underline{\text{H}}_2$-CH=CH$_2$), 4.12 (broad d, 1H, H-4, $J_{4,3}$ = 2.50 Hz), 3.91 (broad d, 1H, H-4', $J_{4',3'}$ = 2.5 Hz), 3.80 - 3.54 (m, 9H, remaining ring hydrogens except H-2), 3.0 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 10.0 Hz).

^{13}C-nmr (D_2O) δ: 134.11 (-O-CH$_2$-$\underline{\text{C}}$H=CH$_2$), 119.25 (O-CH$_2$-CH=$\underline{\text{C}}$H$_2$), 105.23 (C-1'), 102.90 (C-1), 83.74 (C-3), 75.59, 75.38 (C-5, C-5'), 73.17 (C-3), 71.54 (C-2), 71.14 (C-4'), 69.15 (C-4), 68.01 (-O-$\underline{\text{C}}$H$_2$-CH=CH$_2$), 61.56, 61.49 (C-6 and C-6'), 52.42 (C-2).

Allyl 4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-galactopyranoside (40)

Phthalic anhydride (478 mg, 3.4 mmol) was added to a solution of the amine 39 (2.3 g, 6.0 mmol) in pyridine (20 mL). The solution was heated to 60-75°C for 20 min. Triethylamine (593 mg, 5.88 mmol) and more phthalic anhydride (478 mg, 3.4 mmol) were added and the heating was continued for 2 h. Methanol (10 mL) was added and the solution was evaporated to a dry residue which was taken up in 15 mL of a mixture of pyridine-acetic anhydride (2:1). 4-N,N-Dimethylaminopyridine (10 mg) was added and the mixture was heated to 60°C for 12 h. The reaction mixture was then evaporated to a syrup. A solution of the syrup in dichloromethane (150 mL) was washed with ice-cold hydrochloric acid (1 N), aqueous saturated sodium carbonate and finally with water. The solvent was evaporated to dryness and the product was purified by flash chromatography on a column of silica gel using ethyl acetate-n-hexane-acetonitrile (2:2:0.5). Evaporation of the first main fraction left a foam (2.7 g, 62% yield) homogeneous on tlc. An analytical sample was prepared by crystallizing a small quantity of the foam from ethanol; m.p. 165-166°C, $[\alpha]_D^{25}$ +16.0° (c 1.06, $CHCl_3$). A comparison of the ^{1}H-nmr spectrum of the analytical sample with that of the product before crystallization indicated the latter to be essentially pure and therefore it was used as such in the next step.

^{1}H-nmr ($CDCl_3$) (Fig. 29) δ: 7.94 - 7.88 (m, 4H, phthalimido), 5.76 - 5.64 (m, 1H, $-O-CH_2-C\underline{H}=CH_2$), 5.50 (broad d, 1H, H-4, $J_{4,3}$ = 3.0 Hz), 5.27 (broad d, 1H, H-4', $J_{4',3'}$ = 3.0 Hz), 5.18 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 5.03 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.8 Hz), 5.18 - 5.0 (m, 2H, $O-CH_2-CH=C\underline{H}_2$), 4.80 (dd, 1H, H-3', $J_{3',2'}$ = 10.8 Hz, $J_{3',4'}$ = 3.0 Hz), 4.76 (dd, 1H, H-3, $J_{3,2}$ = 10.8 Hz, $J_{3,4}$ = 3.0 Hz), 4.56 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 10.8 Hz), 4.44 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.24 (dd, 1H, H-6a, $J_{6a,5}$ = 5.25 Hz, $J_{6a,6b}$ = 12.0 Hz), 4.12 (dd, 1H, H-6b, $J_{6b,5}$ = 6.75 Hz, $J_{6b,6a}$ = 12.0 Hz), 4.08 - 3.92 (m, 3H, H-5, H-6'a, H-6'b), 3.78 (m, 1H, H-5'), 2.22 - 2.08, 1.54 (6 x s, 18H, 6 x $C\underline{H}_3$ of acetyls).

^{13}C-nmr ($CDCl_3$) δ: 170.54, 170.27, 170.00, 169.02 (8 lines expected, 6 lines observed, 8 x $C=O$), 134.37, 131.70, 123.72, 123.38 (phthalimido carbons), 133.43 ($O-CH_2-\underline{C}H=CH_2$), 117.68 ($-O-CH_2-CH=\underline{C}H_2$), 100.72 (C-1'), 97.46 (C-1), 73.52 (C-3), 71.55 (C-5'), 70.93 (C-5), 70.85 (C-3'), 69.89 (C-2'), 68.73, 68.54 (C-4' and C-4), 66.73 ($-O-\underline{C}H_2-CH=CH_2$), 62.47, 60.88 (C-6' and C-6), 52.62 (C-2), 20.83, 20.76, 20.63, 20.44, 20.00 (6 x $\underline{C}H_3$ of acetyls).

Anal. calcd. for $C_{35}H_{41}NO_{18}$: C 55.05, H 5.41, N 1.83; found: C 54.98, H 5.40, N 1.85

Allyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-galactopyranoside (**42**)

The transformation of the azide **41** to **42** was carried out as described for the preparation of compound **40** from **38**. After purification on a column of silica gel, the title compound was obtained in 45% yield as a foam which crystallized from methanol; m.p. 165-167°C, $[\alpha]_D^{25}$ +19.0° (*c* 1.0).

^{1}H-nmr ($CDCl_3$) (Fig. 30) δ: 7.96 - 7.40 (m, 9H, aromatic), 5.72 - 5.62 (m, 2H, $-O-CH_2-C\underline{H}=CH_2$, benzylic), 5.30 (broad d, 1H, H-4', $J_{4',3'}$ = 3.0 Hz), 5.20 - 5.00 (m, 4H, H-2', H-1, $-O-CH_2-CH=C\underline{\underline{H}}_2$), 4.82 (dd, 1H, H-3', $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.0 Hz), 4.78 (broad d, 1H, H-3, $J_{3,2}$ = 6.0 Hz), 4.56 (d, 1H, H-1', J = 8.0 Hz), 4.40 - 4.36 (m, 2H, H-4, H-6a), 4.28 (m, 1H, $-O-C\underline{\underline{H}}_2-CH=CH_2$), 4.14 - 4.02 (m, 4H, H-6b, H-6'a, H-6'b, $-O-C\underline{\underline{H}}_2-CH=CH_2$), 3.8 (broad t, 1H, H-5', $J_{5',6'}$ = 3.8 Hz), 3.60 (broad s, 1H, H-5), 2.2 - 1.4 (4 x s, 4 x acetyls).

^{13}C-nmr ($CDCl_3$) δ: 170.26, 170.03, 169.15, 168.70, 167.17 (6 lines expected, 5 lines observed, 6 x $\overset{|}{\underset{|}{C}}=O$), 137.85, 134.30, 131.81, 131.71, 128.88, 126.43, 123.70, 123.24 (aromatic carbons), 133.78 ($-O-CH_2-\underline{C}H=CH_2$), 117.23 ($-O-CH_2-CH=\underline{\underline{C}}H_2$), 101.46 (C-1'), 100.93 (benzylic carbon), 97.43 (C-1), 75.67, 74.86 (C-3 and C-4), 71.04 (C-5'), 70.90, 69.26, 69.16, 68.72, 66.97, 66.82, 61.34, 51.70 (remaining carbons of sugar units and aglyconic carbon).

Anal. calcd. for $C_{38}H_{41}NO_{16}$: C 59.43, H 5.38, N 1.82, O 33.35; found: C 59.34, H 5.42, N 1.74, O 33.31.

4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-galactopyranose (**44**)

A solution of compound **40** (1.50 g, 1.96 mmol) in ethanol-benzene-water (7:3:1, 200 mL) containing tris-triphenylphosphine rhodium (III) chloride (200 mg, 0.432 mmol) and 1,4-diazabicyclooctane (66 mg, 0.59 mmol) was refluxed for 24 h. The progress of the reaction was monitored in ^{1}H-nmr by observing the disappearance of the multiplet at 5.7 ppm (Fig. 31). Since no change in composition of the reaction mixture could be observed at 24 h, the solvents were evaporated to dryness to leave a foamy residue which was dissolved in aqueous acetone (90%, 50 mL) containing mercuric chloride (5.0 g, 18.48 mmol) and mercuric oxide (25 mg). After stirring at room temperature for 90 min, the reaction mixture was diluted with water (50 mL) and the product was extracted with chloroform (2 x 75 mL). The chloroform extract was washed with aqueous potassium iodide solution (10%, 2 x 50 mL) and then with water (50 mL). The solvent was evaporated to dryness and applied on a short silica gel column. Evaporation of the main fraction left a foam homogeneous on tlc (1.20 g, 84.6% yield).

4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide (**45**)

Vilsmeier bromide (720 mg, 3.31 mmol) was added to a cooled solution (0°C) of the crude compound 44 (1.2 g, 1.65 mmol) in dichloromethane (15 mL). *sym*-Collidine (440 µL, 3.63 mmol) was added in drops to the reaction mixture and stirred at 0°C for 6 h and then at room temperature for 18 h. The reaction mixture was then washed with ice-cold water, dilute hydrochloric acid, saturated aqueous sodium bicarbonate and finally with water. The solvent was evaporated to leave a foamy material (1.063 g, 82% yield) which was a mixture of anomeric bromides as evidenced from its ^{1}H-nmr spectrum (Fig. 32).

^{1}H-nmr ($CDCl_3$) (Fig. 32) δ: 6.5 (d, H1α , $J_{1\alpha,2\alpha}$ = 3.5 Hz), 6.1 (d, H-1β, $J_{1\beta,2\beta}$= 9.0 Hz), 5.66 (broad d, H-4α, $J_{4\alpha,3}$ = 3.0 Hz), 5.44 (broad d, H-4β, $J_{4\beta,3}$ = 3.0 Hz), 5.38 (dd, H-3α, $J_{3\alpha,2}$ = 10.5 Hz, $J_{3\alpha,4}$ = 3.0 Hz), 5.16 (broad d, H-4'β, $J_{4'\beta,3'}$ = 3.0 Hz), 4.94 (dd, H-2β, $J_{2\beta,1}$ = 9.0 Hz, $J_{2\beta,3}$ = 10.5 Hz), 4.68 (dd, H-3'β, $J_{3'\beta,2}$ = 10.5 Hz, $J_{3'\beta,4'}$ = 3.0 Hz), 4.60 (dd, H-3β, $J_{3\beta,2}$ = 10.5 Hz, $J_{3\beta,4}$ = 3.0 Hz), 4.30 (d, H-1'β, $J_{1'\beta,2'}$ = 8.0 Hz), 4.26 - 3.66 (remaining hydrogens of sugar units), 2.18 - 1.60 (CH_3 of acetyls).

8-Methoxycarbonyloctyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,di-*O*-acetyl-6-*O*-benzoyl-4-*O*{4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-galactopyranosyl}-β-D-galactopyranosyl]-β-D-glucopyranoside (**46**)

4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide (**45**)(531 mg, 0.648 mmol) in nitromethane (5 mL) was added to a solution of the alcohol **20** (465 mg, 0.562 mmol), *sym*-collidine (86 μL, 0.648 mmol) and silver triflate (167 mg, 0.648 mmol) in dry nitromethane (5 mL) cooled to -25°C. The mixture was stirred at -25°C for 6 h and then at room temperature for 12 h. The solids that were deposited after the addition of dichloromethane (30 mL) was removed by filtration and the filtrate was washed with sodium thiosulphate solution, cold water, aqueous HCl and sodium bicarbonate solution. Solvent removal after drying left a foam which was purified by HPLC on a silica gel column, using ethyl acetate-n-hexane-acetonitrile (2:2:0.75). Evaporation of the second main fraction left a foam (548 mg, 62% yield) homogeneous in tlc. $[\alpha]_D$ -8.3° (*c* 1.6, CHCl$_3$0.

^{1}H-nmr ($CDCl_3$) (Fig. 33) δ: 8.06 - 7.46 (broad m, 9H, phthalimido, benzoate), 5.44 (broad d, 1H, H-4", $J_{4'',3''}$ = 3.5 Hz), 5.24 (broad d, 1H, H-4"' , $J_{4''',3'''}$ = 3.5 Hz), 5.12 (d, 1H, H-1", $J_{1'',2''}$ = 8.0 Hz), 5.12 (t, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9.0 Hz), 5.0 (dd, 1H, H-2"' , $J_{2''',1'''}$ = 8.0 Hz, $J_{2''',3'''}$ = 10.5 Hz), 4.86 - 4.74 (m, 4H, H-2, H-3', H-3", H-3"'), 4.70 (dd, 1H, H-6'a, $J_{6'a,5'}$ = 7.0 Hz, $J_{6'a,6'b}$ = 11.0 Hz), 4.53 - 4.30 (m, 6H, H-2", H-2', H-1"', H-1, H-6a, H-6'b), 4.24 (d, 1H, H-1', J = 8.0 Hz), 4.18 - 4.08 (m, 2H, H-4', H-6"a), 4.03 - 3.86 (m, 5H, H-6"'a, H-6"'b, H-6b, H-6"b, H-5"), 3.78 - 3.70 (m, 3H, H-5', H-5"', -O-C$\underline{H}_2$ of aglycon), 3.64 (t, 1H, H-4, $J_{4,5} = J_{4,3}$ = 9.0 Hz), 3.48 (m, 1H, H-5), 3.38 (m, 1H, -O-CH_2 of aglycon), 2.28 (t, 2H, -C$\underline{H}_2$$COOCH_3$), 2.12 - 1.2 (45H, 11 x $\underline{C}H_3$ of acetyls, 12 aglyconic hydrogens).

^{13}C-nmr ($CDCl_3$) δ: 174.24, 170.59, 170.31, 170.24, 170.03, 169.94, 169.47, 169.34, 169.04, 168.05, 166.92, 166.28 (15 x C=O, 15 lines expected, 12 lines observed), 134.50, 134.03, 133.38, 131.73, 131.61, 129.95, 129.71, 128.72, 125.22, 124.67 (phthalimido, benzoate carbons), 100.97, 100.88, 100.30, 98.37 (C-1, C-1', C-1"', C-1"), 75.33, 72.98, 72.83, 72.30, 72.24, 72.01, 71.74, 70.92, 70.87, 70.04, 69.48, 68.72, 68.60, 66.82, 63.26, 62.41, 62.06, 60.97, 52.51 (20 lines expected, 19 lines observed, remaining carbons of sugar units and the aglyconic carbon), 51.0 - 19.59 (16 lines).

8-Methoxycarbonyloctyl 4-O-[4-O-{2-deoxy-3-O-(β-D-galactopyranosyl)-2-phthalimido-β-D-galactopyranosyl}-β-D-galactopyranosyl]-β-D-glucopyranoside (47)

Freshly prepared sodium methoxide in methanol (0.5 mL, 0.5 mmol) was added to a solution of compound 46 (548 mg, 0.357 mmol) in anhydrous methanol (25 mL) and stirred at room temperature under dry nitrogen atmosphere for 4 h. After neutralization with IRA-120H$^+$ resin, the solution was evaporated to get a colorless residue (350 mg, 91.6% yield).

^{1}H-nmr (CD_3OD) δ: 8.1 - 7.81 (m, 4H, phthalimido), 5.23 (d, 1H, H-1", $J_{1",2"}$ = 8.0 Hz), 4.93 (dd, 1H, H-3", $J_{3",2"}$ = 10.0 Hz, $J_{3",4"}$ = 3.0 Hz), 4.52 - 4.35 (m, 4H, H-1, H-2", H-1', H-1"'), 4.1 - 3.30 (remaining pyranose ring hydrogens and $-O-CH_2-C_6$ aglycon, except H-2'), 3.04 (dd, 1H, H-2', $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 9.0 Hz), 2.41 (t, 2H, $-C\underline{H}_2COOCH_3$), 1.64 and 1.33 (remaining hydrogens of aglycon).

8-Methoxycarbonyloctyl 4-O-[4-O-{2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-galactopyranosyl}-β-D-galactopyranosyl]-β-D-glucopyranoside (9)

A solution of compound 47 (350 mg, 0.357 mmol) and 95% hydrazine hydrate (~120 μL) in methanol (20 mL) was refluxed for 8 h. Solvent removal left a syrup which was redissolved in methanol (5 mL) containing acetic anhydride (1 ML) and stirred at room temperature for 3 h. The solution was then evaporated to dryness and dissolved in water (5 mL). The precipitate formed was filtered and the solution was concentrated to 2 mL and filtered through a P-2 Biogel column and eluted with water-ethanol (9:1). Lyphophilization of the first main fractions gave a colorless powder (215 mg, 61.3% yield), $[\alpha]_D^{25}$ -9.2 (c, 0.3).

^{1}H-nmr (D_2O, HOD = 4.8) (Fig.34 and Table 4) δ: 4.64 (d, 1H, H-1", $J_{1",2"}$ = 8.2 Hz, 4.42 (m, 3H, H-1, H-1', H-1‴), 4.11 (broad d, 1H, H-4', $J_{4',3'}$ = 2.2 Hz), 4.05 (broad d, 1H, H-4", $J_{4",3"}$ = 2.2 Hz), 3.95 (dd, 1H, H-2", $J_{2",1"}$ = 8.0 Hz, $J_{2",3"}$ = 10.5 Hz), 3.90 - 3.50 (H, remaining ring protons and -O-CH_2 of aglycon, except H-2' and H-2), 3.55 (dd, 1H, H-2', $J_{2',1'}$ = 8.2 Hz, $J_{2',3'}$ = 10.1 Hz), 3.23 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 8.2 Hz), 2.4 (t, 2H, CH_2-$COOCH_3$), 2.1 (3H, CH_3 of N-acetyl), 1.68 - 1.38 (remaining 12H of aglycon).

^{13}C-nmr (D_2O)(Fig. 35 and Table 5) δ: 178.21 ($-\underline{C}OOCH_3$), 175.3 ($-NH\underline{C}OCH_3$), 105.16 (C-1"), 103.52 (C-1'), 102.75 (C-1"), 102.56 (C-1), 80.2 (C-3"), 79.3 (C-4), 76.5 (C-4'), 75.51 (C-5‴), 75.27 (C-5"), 75.07 (C-5), 75.00 (C-5', C-3), 73.36 (C-2), 73.20 (C-3'), 73.09 (C-3‴), 71.67 ($O-\underline{C}H_2$ of aglycon), 71.25 (C-2'), 71.14 (C-2‴), 69.17 (C-4‴), 68.5 (C-4"), 61.5 (C-6‴, C-6"), 61.2 (C-6'), 60.8 (C-6), 52.5 ($-COO\underline{C}H_3$), 52.1 (C-2"), 34.2 - 22.9 (7 signals, carbons in aglycon and $-NH-CO\underline{C}H_3$).

n-Propyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranoside (49)

A solution of compound 38 (46 mg, 0.11 mmol) in methanol (4 mL) containing Palladium on carbon (10%, 10 mg) was stirred under an atmosphere of hydrogen for 3h. The reaction mixture was filtered and evaporated to dryness. The residue (38 mg) was taken up in a mixture of methanol-acetic anhydride (10:1, 1 mL) and stirred at room temperature for 3 h. Examination of the reaction mixture at this time (EtOAc-EtOH-H_2O, 16:8:5) indicated the presence of a single compound. It was then evaporated to a dry residue which was redissolved in water and freeze-dried. This afforded a colorless material (40 mg, 0.09 mmol, 89% yield) which was identified as the desired compound by ^1H- and ^{13}C-nmr. $[\alpha]_D^{25}$ 8.0° (c, 0.9).

^1H-nmr (D_2O)(see Table 4) δ: 4.53 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.46 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1'), 4.01 (dd, 1H, $J_{2,3}$ - 11.2 Hz, 1H, H-2), 3.95 - 3.51 (remaining sugar hydrogens and aglyconic hydrogens), 2.02 (s, 3H, -NHCO-CH_3), 1.56 (m, 2H, -O-CH_2-$C\underline{H}_2$-CH_3), 0.88 (t, J = 7.3 Hz, 3H, O-CH_2-CH_2-$C\underline{H}_3$).

^{13}C-nmr (D_2O)(see Table 5) δ: 105.06 (C-1'), 101.56 (C-1), 80.19 (C-3), 75.26 (C-5'), 75.00 (C-5), 72.78 (C-3'), 72.45 ($O-\underline{C}H_2$ of aglycon), 70.92 (C-2'), 68.88 (C-4'), 68.32 (C-4), 61.28 (C-6'), 61.19 (C-6), 51.63 (C-2), 22.51, 22.35 ($-O-CH_2-\underline{C}H_2-CH_3$, $-NH-\overset{O}{C}-\underline{C}H_3$), 9.85 ($O-CH_2-CH_2-\underline{C}H_3$).

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